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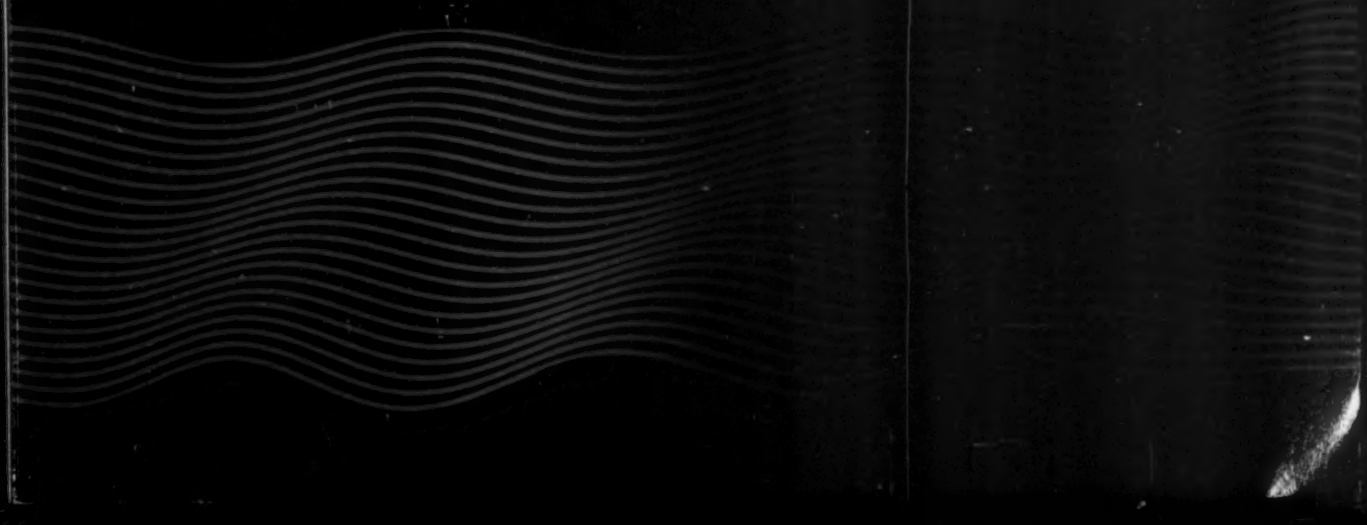

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Timing of Sea-Run Brown Trout (*Salmo trutta*) Smolt Migration: Effects of Climatic Variation

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Bohlin, T., C. Dellefors, and U. Faremo. 1993. Timing of sea-run brown trout (*Salmo trutta*) smolt migration: effects of climatic variation. Can. J. Fish. Aquat. Sci. 50: 1132–1136.

By trapping seaward migrating smolts of brown trout (*Salmo trutta*), we analyzed the timing of the run in a small stream in southwestern Sweden (58°N) during 1984–90 in relation to environmental factors. Ninety percent of the smolts were captured during a period of 28.7 d (SD 5.9), with median time ranging from April 26 to May 17. Using polynomial multiple regression, we found a positive relationship between the probability of migration per day and the number of degree-days, change in water level, temperature change during the preceding week, and mean annual fish length. Forty-seven percent of the variation was explained. The predicted probabilities were used to calculate the population fraction migrating per day for the seasons 1984–90. There was a close agreement between the observed and expected daily fraction of migrants. The model, with parameter values based on the observations from 1984–90, was also used to predict the daily migration in 1991. The result indicated that the model accurately predicts the smolt run ($R^2 = 0.40$).

En piégeant des smolts de truite brune (*Salmo trutta*) pendant la migration vers la mer, nous avons analysé le calendrier de l'avalaison dans un petit cours d'eau du sud-ouest de la Suède (58°N), pendant 1984–1990, en fonction de facteurs environnementaux. Quatre-vingt-dix pourcent des smolts ont été capturés sur une période 28,7 jours (écart type, 5,9), la période moyenne s'étendant du 26 avril au 17 mai. À l'aide d'une régression multiple polynômiale, nous avons trouvé une relation positive entre la probabilité de migration quotidienne et le nombre de degrés-jour, les changements du niveau de l'eau, l'évolution de la température la semaine précédente et la longueur moyenne annuelle du poisson. Quarante-sept pourcent de la variation a pu être expliquée. Les probabilités prédites ont servi à calculer la fraction de la population qui migre par jour pour les saisons 1984–1990. Il y avait une concordance étroite entre la fraction quotidienne observée et prévue de poissons en migration. Le modèle, avec des valeurs paramétriques fondées sur les observations de 1984 à 1990, a aussi servi à prédire la migration quotidienne en 1991. Le résultat indique que le modèle prédit avec exactitude l'avalaison de smolts ($R^2 = 0,40$).

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The smolt transformation of salmonids, a process that is similar among *Salmo* and *Oncorhynchus* species, includes development of saltwater resistance and preference and is controlled by photoperiod (Hoar 1976; Wedemeyer et al. 1980; Folmar and Dickhoff 1981). The result is migration at a variable age but rather uniform size during a relatively short period in spring (Hoar 1976).

The timing of the smolt migration is of applied as well as general interest and has been treated mainly from two different but interrelated viewpoints. One is based on life-history theory and emphasizes individual variation in age or time of migration as a result of differences in body size, growth rate, and mortality (Bohlin et al. 1993). The second line of reasoning stems from the observation that migration often is temporally varying within and among seasons, and the question has been to explain and interpret this variation in terms of environmental, mainly climatic, factors. Temperature and/or water flow have been associated with the smolt migration with varying success (Solomon 1978, 1982; Ruggles 1980; Northcote 1984; Jonsson and Ruud-Hansen 1985; Thorpe 1988). Quantitative predictions relating the migration timing to external factors are, however, scarce. An exception is Jonsson and Ruud-Hansen (1985). They managed to explain most of the variation in median time of salmon smolt migration in a 8-yr period by the temperature during a period prior to migration. They found no effects of water level on the median time.

We quantified the relationship between some climatic conditions and the smolt migration timing of a wild sea-run brown trout (*Salmo trutta*) population in a small stream in Sweden. Using polynomial regression, we estimated the effect of number of degree-days, change in water level, temperature change, and mean smolt body length on migration probability per day of the season.

Model Formulation and Methods

During 1984–91, descending brown trout smolts were caught in a trap in Norumsån, a small stream (mean width 2.4 m, length 8.5 km) in southwestern Sweden (58°N). The grid mesh in the trap was 10 mm which retained fish >95 mm total body length. The trap was set in operation as soon as ice conditions and water level permitted (mid-March to mid-April) and was closed at the beginning of June. The catch was recorded daily during the peak run together with total body length to the nearest millimetre, morning water temperature (±0.2°C), and water

level (±0.5 cm). In all, the catch was recorded on 220 occasions during the 8-yr period.

From the trapping record 1984–90, we used the total catch for each year and estimated the migration probability p for each day as (number caught that day)/(number of migrants still in the stream on the day before). If the trap had not been visited on day x but on day $x + 1$, we assumed that the numbers of migrants on day x and day $x + 1$ were distributed according to equal migration probability.

Formulation of the model required choosing variables and making assumptions about how these variables interact and affect the probability of migration p. The physiological processes leading to migration, e.g., anabolic processes that restore the nutritional state after the winter conditions, may require time in operation and are likely to be temperature dependent. We therefore assumed that the probability of migration was positively affected by the number of degree-days (DD) measured from the onset of smoltification in early spring. As rising water level has been suggested to promote migration (Solomon 1978, 1982), we also included the change in water level (DWL). Temperature, or temperature change (DT), might also be associated with migration probability. As absolute temperature is already incorporated in DD, we included DT in the model. Finally, the average body size of smolts may also affect the migration probability; large smolts tend to migrate earlier (Bohlin et al. 1993) and thereby require fewer DD before migrating. The average mean smolt length L was therefore also included.

We used the following polynomial models:

$$(1a)\quad p = \text{year} + a + \text{DD}(b_1 + b_2\,\text{DWL} + b_3\,\text{DT} + b_4 L)$$

$$(1b)\quad p = a + \text{DD}(b_1 + b_2\text{DWL} + b_3\text{DT} + b_4 L)$$

where a is the intercept and b_1 to b_4 are the regression coefficients. In (1a), we used year as a class variable (analysis of covariance model) to test the year effect. A significant year effect would mean that the seasons are characterized by additional variables not included in the regression. The reason for choosing a polynomial relationship was that the effects of variables DWL, DT, and L on migration probability are likely to depend on DD; for low values of DD, viz. in the early season, the effect of the other variables is also probably low.

Since the p values calculated from the trapping record were based on different numbers of observations, they were weighted into the model. Usually the weighting factor is the inverse of the sampling variance (e.g., Seber 1973, p. 6) ($= n/(p(1 - p))$ for a binomial distribution). This leads to awkward results when $p = 1$ or 0; we therefore used the number of migrants n still left in the stream as the weighting factor. To estimate the parameters a and b_1 to b_4 in (1b), we used the pooled observations for the years 1984–90.

The number of DD was calculated as DD above 4°C from March 1. DWL (centimetres) from day $x - 1$ to day x was treated in the following way. As the response to DWL might remain for some time after the change, we used as DWL on day x the largest of the values DWL_x, DWL_{x-1}, and DWL_{x-2}, viz. the largest daily DWL during the last 3 d. DT was assumed to be the change in temperature over the last week. The values for L (millimetres total length) used were the annual means of all smolts caught.

The parameters in (1a) and (1b) were estimated using the GLM process in SAS Institute Inc. (1982).

Probability p in (1b) was the predicted probability of migration for migrants still remaining in the stream, ranging from a low value in the early run to 1 for the last day's observation. These values were transformed into the population fraction P_x predicted to migrate each day x. Parameter P_x is the joint probability of not having left the stream on any of the preceding days and the migration probability p on day x. Hence, we calculated the proportion P of migrants on day x from the predicted values of p as

$$(2)\quad P_x = p_x \cdot (1 - p_{x-1}) \cdot (1 - p_{x-2}) \cdot \ldots \cdot (1 - p_0).$$

As a result of the linearity in model (1), occasional predicted values of p in the early season were negative; these were set to 0 in (2). We then compared the prediction from (2) with our observed values of P, estimated as (number captured on day x)/(number of all smolts captured that season).

The estimates based on the period 1984–90 were then used to predict the smolt run during the 1991 season.

Results

On average, 90% of the run occurred during a period of 28.7 d (SD 5.88, $n = 7$), ranging from 22 d in 1988 to 39 d in 1990 (Fig. 1). The mean date (50% run) was May 7 (SD 7.8, $n = 7$), ranging from April 26 in 1990 to May 17 in 1984. Most major peaks coincided with periods with increasing water level.

Annual mean L, standard error, and sample size for the 7 yr of the investigation were as follows:

Year	L	SE	n
1984	132.7	0.339	1059
1985	151.7	0.623	655
1986	146.1	0.654	430
1987	146.4	0.609	655
1988	149.2	1.104	249
1989	136.6	0.794	1599
1990	136.7	0.671	2082

To estimate the partial regression coefficients, we first used model (1a) to test the year effect. This, however, was not significant ($p = 0.14$). We therefore used the model as the polynomial multiple regression (1b).

The estimates of the partial regression coefficients and the values of significance were as follows: $a = -0.0120$, $b_1 = 0.00920$, $b_2 = 0.000107$, $b_3 = 0.000232$, $b_4 = 0.0000740$; $p_a = 0.20$, $p_{\text{DD}} = 0.0019$, $p_{\text{DD·DWL}} = 0.0001$, $p_{\text{DD·DT}} = 0.0001$, $p_{\text{DD·}L} = 0.0006$, $p_{\text{model}} < 0.0001$, $R^2 = 0.465$.

Approximately half of the variance (47%) in p was thus explained by the model. All variables had significant effects. Of the explained variance, DD accounted for the major part (53%), DD·DVL for 30%, DD·DT for 11%, and DD·L for 6%. By using temperature rather than DT in (1b), the fit decreased to $R^2 = 0.40$. When using temperature instead of DD, however, the fit was substantially reduced ($R^2 = 0.31$).

We concluded that the probability of migration on day x tended to increase with DD. The positive effects of increasing water temperature, rising water level, and increasing mean length of smolt all interacted with DD.

The expected population fraction P_x of migrants per day, from (2), was then compared with our observations. The temporal variation in the run was reasonably well predicted both within and among seasons (Fig. 1). Even in the 1991 run, which

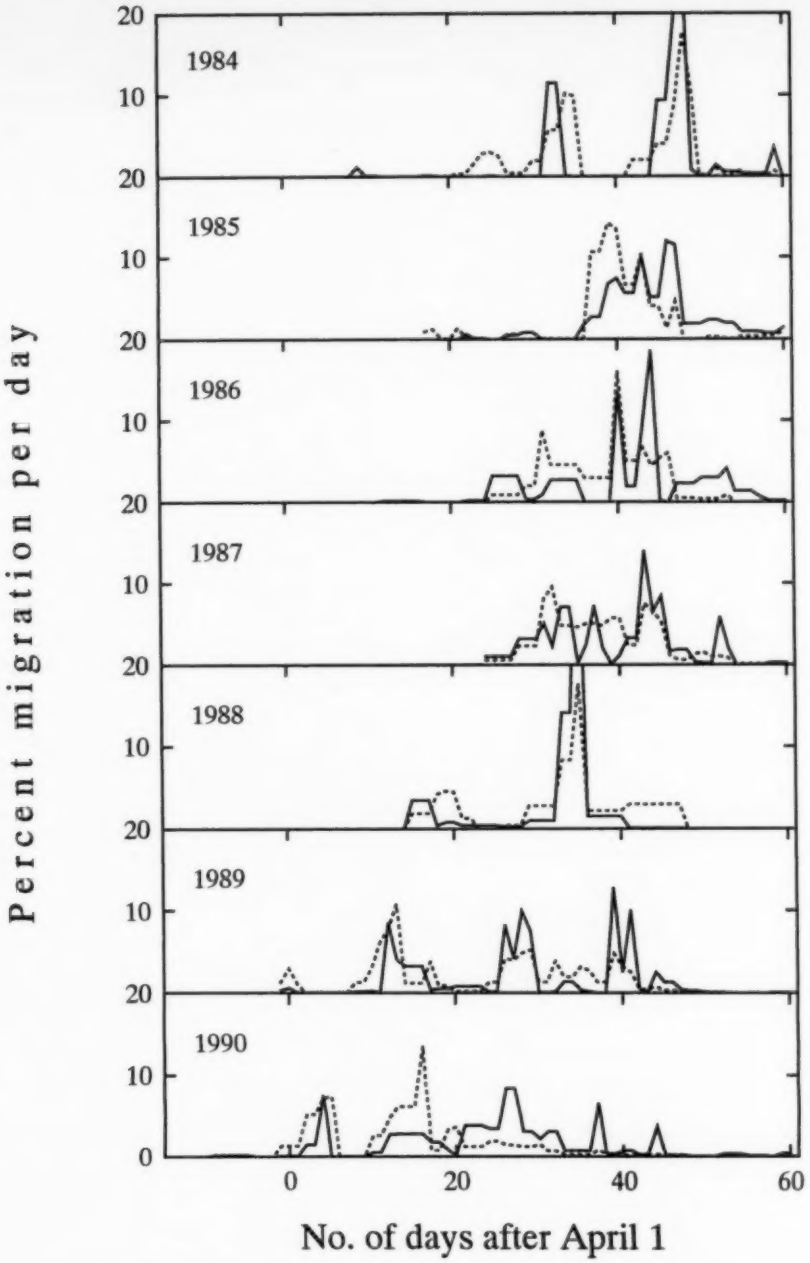

FIG. 1. Observed (solid lines) and predicted (broken lines) smolt run during 1984–90.

was predicted using independent data from 1984–90, the expected migration closely followed the observed (Fig. 2). For these data, the fit to (1b), with the parameter estimates given above, corresponded to $R^2 = 0.40$.

Discussion

The timing of seaward migration in Norumsån, southwestern Sweden, was positively associated with the number of degree-days, change in water level, temperature change, and average body length of smolts. A previous tagging experiment (Bohlin et al. 1993) also revealed that initially bigger smolts tended to migrate earlier than smaller ones. A warm spring is therefore likely to be associated with an early run, particularly in years when fish growth was high the previous year.

One question of practical relevance is: to what extent is the positive relationship between migration probability and smolt size valid also for hatchery-reared smolts? Usually, hatchery

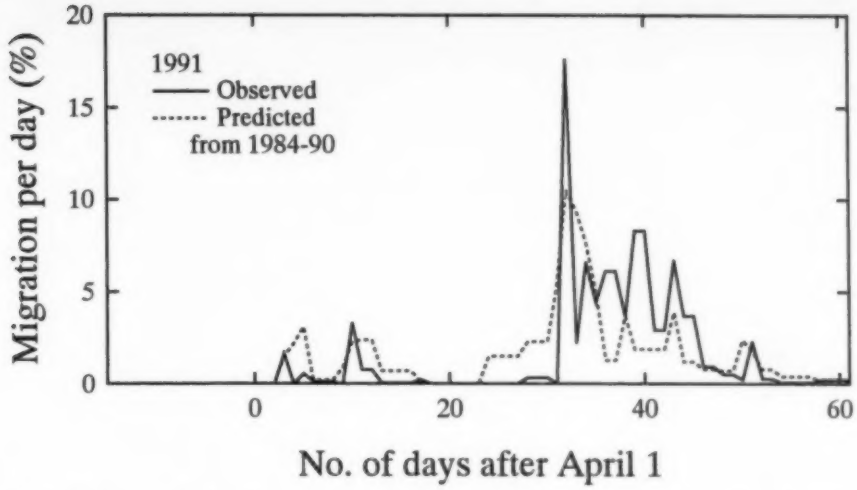

FIG. 2. Observed (solid line) and predicted (from independent data) (broken line) smolt run in 1991.

smolts are much larger than wild ones, and the optimal release date may thus be earlier than the peak in run of wild smolts. As far as we know, there are no published data on comparisons between the migration time of wild smolt and hatchery-reared smolts released before "normal" migration time. Generally, hatchery-reared salmon smolts tend to emigrate immediately after release when stocked during the expected migration period for wild smolts (Doving et al. 1984; Hansen et al. 1984; Kennedy et al. 1984; Hansen and Jonsson 1985). This does not exclude the possibility that they would migrate earlier given that opportunity.

Absolute temperature has often been suggested as a cue for the downstream migration of salmonids (see Ruggles 1980); occurrence of migration as temperature approaches 10°C has been used as a rule of thumb. In our region the figure suggested has been 7°C. We found, however, that absolute temperature was of limited value as a predictor of smolt migration in comparison with degree-days.

The effect of degree-days on probability of migration can be interpreted in two different ways. One is that a smolt, depending on its size, needs to experience a certain number of degree-days before it is physiologically ready for migration. The other is that degree-days, or some variable(s) correlated with this, are used merely as a cue for optimal timing. In this case the degree-days are not "needed" but serve as information. We believe that neither alternative is correct. A combination seems more likely; physiological preparation may be required, at least to some extent, but the question for the individual is the optimal moment to exchange preparation for action. The effect of degree-days may thus be dual, affecting physiological processes as well as serving as a cue of environmental conditions.

The daily numerical variation of the smolt run was positively associated with changes in temperature and, in particular, with water levels. The latter is to be expected (Solomon 1978, 1982), at least in small streams. Possible reasons are that high or increasing flow facilitates traversing of obstacles and that the turbidity often associated with rising water level reduces predation. Although Jonsson and Ruud-Hansen (1985) found no significant effect of water level on the median migration date in an 8-yr study of wild Atlantic salmon (*Salmo salar*) smolts, they did not analyze the day-to-day variation in number of migrants. Possibly water level, or water level change, could affect day-to-day variation in number of migrants without significantly affecting the date of 50% migration.

Although the migration pattern was reasonably well predicted, it was evident that the observed migration number fluctuated temporally more than expected (Fig. 1 and 2). A reason for this could be the choice of variables and/or the model formulation. Another possibility is that smolts may form schools (Hansen and Jonsson 1985), which implies that the migration probabilities on day x and day $x + 1$ are not independent; if, due to chance, more individuals than expected migrate on day x, then fewer might migrate on day $x + 1$. The general effect of such an autocorrelation is a reduced fit, viz. a larger unexplained variance. One component of the unexplained variance in our model may thus be schooling behaviour.

Our adaptive interpretation is thus that an individual fish's probability of migration is increasing with time or degree-days in a way that is affected by body size. Environmental fluctuation, particularly in water level, creates an additional probability factor through its effects on the cost of migration (e.g., risk of predation, energetic costs). Increasing or decreasing flow may thereby cause an individual to migrate earlier or later than predicted from size and growth alone. Migration in schools may also reduce the risk of predation, and the result is likely to be an increased temporal variation not explained by body size and environmental factors.

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Identification of Pathogenic Fish Bacteria Using the API ZYM System

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The API ZYM system was used to identify the bacterial fish pathogens *Enterococcus seriolicida*, β-hemolytic *Streptococcus* sp., *Renibacterium salmoninarum*, *Aeromonas salmonicida*, *A. hydrophila*, *Pasteurella piscicida*, *Edwardsiella tarda*, *Vibrio anguillarum*, *Pseudomonas fluorescens*, *Flexibacter columnaris*, and *F. maritimus*. As additional tests, Gram stain and the activities of cytochrome oxidase and catalase were examined. The results of API ZYM and the additional tests were coded for easy identification. Several biotypes were demonstrated by *Streptococcus* sp., *V. anguillarum*, and *A. hydrophila*. Other bacteria showed uniform profiles. This system can distinguish each fish pathogen from all other bacterial species examined.

Le système API ZYM a servi à identifier des bactéries pathogènes du poisson, *Enterococcus seriolicida*, *Streptococcus* sp. β-hémolytiques, *Renibacterium salmoninarum*, *Aeromonas salmonicida*, *A. hydrophila*, *Pasteurella piscicida*, *Edwardsiella tarda*, *Vibrio anguillarum*, *Pseudomonas fluorescens*, *Flexibacter columnaris* et *F. maritimus*. Comme tests supplémentaires, on a examiné la coloration de Gram et les activités de la cytochrome oxydase et de la catalase. Les résultats fournis par API ZYM et les tests supplémentaires ont été codés pour faciliter l'identification. On a pu repérer plusieurs biotypes chez *Streptococcus* sp., *V. anguillarum* et *A. hydrophila*. D'autres bactéries présentaient des profils uniformes. Ce système permet de distinguer chaque pathogène de toutes les autres espèces bactériennes examinées.

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Serological procedures for the diagnosis of infectious diseases have been widely used in fish (Schill et al. 1989). Several serodiagnostic procedures are very sensitive and rapid; however, the presence of cross-reactive antigens has often obscured the precise diagnosis of fish disease. Thus, the confirmatory diagnosis of fish disease requires the isolation and identification of each particular pathogen.

Most pathogenic fish bacteria are routinely identified using classical biochemical tests. While accurate, these procedures are often cumbersome because of the wide variety of organisms involved.

Recently, kits to identify pathogenic bacteria in human and veterinary medicine have been developed. In fish, these kits were used for the identification of *Streptococcus* sp. (Hashimoto 1982; Iida et al. 1991), *Vibrio anguillarum* (Kent 1982; Grisez et al. 1991), *Pasteurella piscicida* (Kent 1982), and *Yersinia ruckeri* (Romalde and Toranzo 1991). However, these kits can be used only for the identification of bacteria within a specific taxonomic group. Thus, it is necessary to develop a kit that can be applied to the wide range of pathogenic fish bacteria.

The API ZYM system has been used to detect the activity of 19 different enzymes in samples such as bacteria, tissues, or cells. It has been used to study the enzymatic profiles of streptococci, staphylococci (Humble et al. 1977), *Moraxella* sp. (Frank and Gerber 1981), and *Pseudomonas cepacia* (Poh and Loh 1988). The enzymatic characterization of the pathogenic fish bacteria *Aeromonas hydrophila* (Waltman et al. 1982) and *Renibacterium salmoninarum* (Austin and Austin 1987) using this kit has also been reported. In this study, we attempt to identify the important pathogenic bacteria in aquaculture in Japan using the API ZYM system.

Materials and Methods

Bacterial Strains

Enterococcus seriolicida, *Streptococcus* sp., *R. salmoninarum*, *Aeromonas salmonicida*, *A. hydrophila*, *P. piscicida*, *Edwardsiella tarda*, *V. anguillarum*, *Pseudomonas fluorescens*, *Flexibacter columnaris*, and *F. maritimus* were used in this study (Table 1). All bacteria were suspended in physiological saline (0.85% NaCl) and adjusted to the concentration of McFarland No. 5.

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TABLE 1. Species and sources of fish pathogenic bacterial strains and culture media used in this study.

Bacterial species	Source	Location	No. of strains	Culture media[a]	Temperature (°C)
Enterococcus seriolicida	Yellowtail	Kagoshima	21	BHI	30
Streptococcus sp.	Yellowtail	Kagoshima	12	BHI	30
	Rainbow trout	Miyazaki	5		
	Ayu	Shiga	1		
	Tilapia	Kagoshima	1		
	Sea bream (*Archosargus probatocephalus*)	Kagoshima	4		
	Japanese flounder	Kagoshima	1		
Renibacterium salmoninarum	Coho salmon	Iwate	6	KDM-2	15
Aeromonas salmonicida	Coho salmon	Iwate	2	TS	20
	Charr (*Salvelinus leucomaenis*)	Iwate	4		
Aeromonas hydrophila	Japanese eel (*Anguilla japonica*)	Miyazaki	11	TS	30
Vibrio anguillarum	Coho salmon	Miyagi	10	1TS	25
	Ayu	Shiga	1		
Edwardsiella tarda	Japanese flounder	Kagoshima	6	TS	30
Pasteurella piscicida	Yellowtail	Kagoshima	22	2TS	30
		Miyazaki	8		
Pseudomonas fluorescens	Rainbow trout	Iwate	1	TS	30
Flexibacter columnaris	Rainbow trout	Iwate	1	TY	25
Flexibacter maritimus	Sea bream	Tokushima	1	MTY	25

[a]BHI, brain heart infusion agar; TS, trypto-soya agar; 1TS, trypto-soya agar with 1% NaCl; 2TS, trypto-soya agar with 2% NaCl; MTY, TY agar with 50% seawater.

API ZYM

The API ZYM system (La Balme les Grottes, 38390 Montalieu, France) is capable of detecting and measuring the presence of 19 enzymes. Two drops of each bacterial suspension were added to each of the 20 cupules of one panel. Each panel was incubated wtihin its humidification chamber for 6 h. The incubation temperature for each bacterial suspension is shown in Table 1. After incubation, one drop of reagent A (250 g of Tris, 110 mL of 37% HCl, and 100 g of sodium dodecyl sulfate made up to a final volume of 1000 mL of distilled water) and one drop of reagent B (3.5 g of Fast Blue BB per 1000 mL of 2-methoxyethanol) were added to each cupule. Color reactions were allowed to develop for 5 min, after which the cupules were exposed to a 500-W high-intensity light source for 10 s. The presence of enzymatic activity was determined by comparing the color of the bacterial samples with the manufacturer's reading scale. All test strains were examined twice.

Additional Tests

Gram stain, cytochrome oxidase, and catalase tests were performed as additional tests. Gram stain was conducted using Hucker's method (Hendrickson and Krenz 1991). The cytochrome oxidase and catalase tests were performed using the methods of Cowan (1974).

Bacterial Identification

Each API ZYM result was scored as follows. Nineteen enzymes were divided into seven groups, each composed of three enzymes, with the exception of α-fucosidase (Table 2). If the activity of the first enzyme in each group is positive, the score is 1. If the activity of the second enzyme in each group is positive, the score is 2. If the activity of the third enzyme in each group is positive, the score is 4. No score is given for negative reactions. Finally, the scores for each group are totalled and expressed as a seven-digit number. Thus, the maximum score for each enzymatic test is 7 and the minimum is 0.

TABLE 2. Code method used for the API ZYM profiles and additional tests (e.g. *Pasteurella piscicida*).

Characteristic	Results	No. of codes	Total No. of codes
Rod			
Gram stain	−	0	0
Cytochrome oxidase	+	1	
Catalase	+	2	3
Alkaline phosphatase	+	1	
Butyrate esterase (C4)	+	2	
Caprylate esterase (C8)	+	4	7
Myristate lipase (C14)	−	0	
Leucine arylamidase	+	2	
Valine arylamidase	−	0	2
Cystine arylamidase	−	0	
Trypsin	−	0	
Chymotrypsin	−	0	0
Acid phosphatase	+	1	
Phosphoamidase	+	2	
α-Galactosidase	−	0	3
β-Galactosidase	−	0	
β-Glucuronidase	−	0	
α-Glucosidase	−	0	0
β-Glucosidase	−	0	
N-Acetyl-β-glucosaminidase	+	2	
α-Mannosidase	−	0	2
α-Fucosidase	−	0	0
Pasteurella piscicida	Rod	037203020	

TABLE 3. Enzymatic activities of Gram-positive pathogenic fish bacteria detected with API ZYM. +, 90% or more strains positive; −, 90% or more strains negative; V, 11–89% of strains positive. ES, *Enterococcus seriolicida*; SS, *Streptococcus* sp.; RS, *Renibacterium salmoninarum*.

Enzyme	ES	SS	RS
No. of strains	21	24	6
Alkaline phosphatase	−	+	+
Butyrate esterase (C4)	+	+	+
Caprylate esterase (C8)	+	+	+
Myristate lipase (C14)	−	V (83.3%)	V (33.3%)
Leucine arylamidase	+	+ (95.8%)	+
Valine arylamidase	−	V (58.3%)	−
Cystine arylamidase	V (57.1%)	−	−
Trypsin	−	−	+
Chymotrypsin	+	+	−
Acid phosphatase	+	+	+
Phosphoamidase	+	+	V (83.3%)
α-Galactosidase	−	−	−
β-Galactosidase	−	−	−
β-Glucuronidase	−	−	−
α-Glucosidase	+ (95.2%)	V (70.8%)	+
β-Glucosidase	+	V (41.7%)	−
N-Acetyl-β-glucosaminidase	−	−	−
α-Mannosidase	−	−	+
α-Fucosidase	−	−	−

The results of the additional tests were scored by the same method. The Gram stain is treated as one group; the score is 1 for Gram-positive bacteria and 0 for Gram-negative bacteria. The cytochrome oxidase and catalase tests were in the same group. Cytochrome-oxidase-positive bacteria are given a score of 1; negatives are given a score of 0. For catalase, the score for a positive reaction is 2 and for a negative reaction is 0. Adding in the scores from the additional tests results in a nine-digit number to characterize each bacterial strain.

Results and Discussion

The API ZYM system was useful for identifying many pathogenic bacteria of fishes. *Enterococcus seriolicida* infects yellowtail (*Seriola quinqueradiata*) and was recently described by Kusuda et al. (1991). The API ZYM activities of this bacterium had only two patterns (106243010 and 106243410) (Tables 3 and 4) and the code number was not close to those for other bacteria. Kitao (1982) also examined the biochemical characteristics of *E. seriolicida* and reported similar results except in the fermentation of several sugars, the VP test, and citrate utilization. Using API Strep, Hashimoto (1982) and Iida et al. (1991) also reported that strains of this bacterium showed similar biochemical characteristics.

β-Hemolytic streptococcal infection occurs in yellowtail, rainbow trout (*Oncorhynchus mykiss*, formerly *Salmo gairdneri*), tilapia (*Oreochromis* sp.) (Kitao et al. 1981), ayu (*Plecoglossus altivelis*) (Ohnishi and Jo 1981), and Japanese flounder (Nakatsugawa 1983). The biochemical characterization of β-hemolytic streptococcus strains isolated from each of the fish species shows close similarities, suggesting that the infection is caused by one bacterial strain, identified as a biotype of *Streptococcus iniae* (T. Kitao, personal communication). Iida et al. (1991), using API Strep, reported that strains of β-hemolytic *Streptococcus* sp. isolated from ayu and tilapia had similar characteristics, although this bacterium was not classified like any *Streptococcus* species previously reported. In this study, 31 β-hemolytic *Streptococcus* sp. isolated from yellowtail showed several patterns. The 31 strains of β-hemolytic *Streptococcus* sp. were classified into 14 types by the API ZYM test (Tables 3 and 4). Prior to this study, these bacteria had only been examined only by Gram stain, cytochrome oxidase, catalase, hemolysin, and an agglutination test using antiserum to β-hemolytic *Streptococcus* sp. isolated from ayu. The results of the API ZYM test suggest that these infections are caused by several types of β-hemolytic *Streptococcus* sp.

Renibacterium salmoninarum is the causative agent of bacterial kidney disease in salmonid fish. The biological characteristics of isolates of this bacterium from several regions are similar (Bruno and Monro 1986). In the API ZYM test described here, only three types of *R. salmoninarum* were detected (127223440, 127321440, and 127323440). Austin and Austin (1987) reported that 48 *R. salmoninarum* isolates had alkaline phosphatase, caprylate esterase (C8), leucine arylamidase, trypsin, acid phosphatase, phosphoamidase, β-glucosidase, and α-mannosidase. Our *R. salmoninarum* strains isolated in Japan showed a positive reaction for butyrate esterase (C4), in addition to those cited by Austin and Austin (1987). This difference in butyrate esterase (C4) activity may reflect a geographic difference in types of bacterial enzymes present.

Aeromonas salmonicida is one of the most studied fish pathogens. It can be separated into three subspecies, *salmonicida*, *achromogenes*, and *masoucida*, that differ in their biochemical characteristics such as brown water-soluble pigment formation and sucrose and mannitol fermentation (Popoff 1984). In this study, we used six strains of *A. salmonicida* ssp. *salmonicida* and no differences were detected using the API ZYM test (Table 5, 037723030). Thus, this method is very useful for identification of this species, although other subspecies must be examined.

Aeromonas hydrophila has been described as an etiological agent, not only in diseases of fish (Austin and Austin 1987) but also in those of amphibians, reptiles (Shotts et al. 1972), and humans (Von Graevenitz and Mensch 1968). This bacterium is

TABLE TABLE 4. List of pathogenic fish bacteria with code numbers as determined with API ZYM and additional tests (see text).

Bacterial species	Shape	Code No. (no. of bacteria)	Bacterial species	Shape	Code No. (no. of bacteria)
Enterococcus seriolicida	Cocci	106243010 (1)			037703030 (1)
		106243410 (8)			037703120 (2)
		106253410 (12)			037703330 (1)
Streptococcus sp.	Cocci	107143400 (1)	*Aeromonas hydrophila*	Rod	037303030 (1)
		107243010 (1)			037323030 (1)
		107243410 (1)			037323120 (1)
		107253410 (2)			037323330 (3)
		107343000 (1)			037643510 (1)
		107343400 (4)			037703020 (1)
		107343410 (3)			037723130 (1)
		107353410 (1)			037723330 (1)
		107743000 (2)			037723430 (1)
		107743010 (1)	*Aeromonas salmonicida*	Rod	037343030 (6)
		107743400 (2)	*Edwardsiella tarda*	Rod	027203020 (6)
		107753000 (2)	*Pasteurella piscicida*	Rod	037203020 (30)
		107753010 (2)	*Pseudomonas fluorescens*	Rod	037623000 (1)
		107753400 (2)	*Renibacterium salmoninarum*	Rod	127223440 (4)
Vibrio anguillarum	Rod	037603120 (3)			127321440 (1)
		037603520 (1)			127323440 (1)
		037702420 (1)	*Flexibacter columnaris*	Rod	037633000 (1)
		037703020 (4)	*Flexibacter maritimus*	Rod	037713000 (2)

TABLE 5. Enzymatic activities of Gram-negative pathogenic fish bacteria detected with API ZYM. +, 90% or more strains positive; −, 90% or more strains negative; V, 11–89% of strains positive. AS, *Aeromonas salmonicida*; AH, *Aeromonas hydrophila*; VA, *Vibrio anguillarum*; PP, *Pasteurella piscicida*; ET, *Edwardsiella tarda*; PF, *Pseudomonas fluorescens*; FC, *Flexibacter columnaris*; FM, *Flexibacter maritimus*.

Enzyme	AS	AH	VA	PP	ET	PF	FC	FM
No. of strains	6	11	11	30	6	1	1	2
Alkaline phosphatase	+	+	+	+	+	+	+	+
Butyrate esterase (C4)	+	+	+	+	+	+	+	+
Caprylate esterase (C8)	+	+	+	+	+	+	+	+
Myristate lipase (C14)	+	+ (90.9%)	V (69.2%)	−	−	+	−	+
Leucine arylamidase	+	+	+	+	+	+	+	+
Valine arylamidase	−	V (45.4%)	+	−	−	−	+	+
Cystine arylamidase	−	− (9.1%)	−	−	−	−	+	−
Trypsin	−	V (72.7%)	−	−	−	+	+	−
Chymotrypsin	+	− (9.1%)	−	−	−	−	−	+
Acid phosphatase	+	+	+ (92.3%)	+	+	+	+	+
Phosphoamidase	+	+	+	+	+	+	+	−
								−
α-Galactosidase	−	−	−	−	−	−	−	−
β-Galactosidase	−	V (63.6%)	V (53.8%)	−	−	−	−	−
β-Glucuronidase	−	V (36.4%)	− (7.7%)	−	−	−	−	−
α-Glucosidase	−	V (18.1%)	V (15.4%)	−	−	−	−	−
β-Glucosidase	+	V (81.9%)	V (15.4%)	−	−	−	−	−
N-Acetyl-β-glucosaminidase	+	+ (90.9%)	+	+	+	−	−	−
α-Mannosidase	−	−	−	−	−	−	−	−
α-Fucosidase	−	−	−	−	−	−	−	−

usually identified by Gram-negative fermentative rods that are motile and capable of growth at 37°C, catalase and cytochrome oxidase positive, and O/129 resistant. However, other biochemical characteristics of bacteria identified as *A. hydrophila* are very variable, and it is difficult to make generalizations about their exact taxonomic status. Our results showed nine profiles in the 11 strains examined (Table 4). Waltman et al. (1982) reported that all *A. hydrophila* isolated from fish showed positive reactions to caprylate esterase (C8), leucine arylamidase, acid phosphatase, phosphoamidase, *N*-acetyl-β-glucosaminidase, and β-galactosidase. In this study, caprylate esterase (C8), leucine arylamidase, acid phosphatase, phosphoamidase, and *N*-acetyl-β-glucosaminidase were positive in all strains examined. The only difference was the lack of β-galactosidase activity. Although many API ZYM profiles seemed to appear in *A. hydrophila* strains isolated from fish, only the main enzymatic activities caprylate esterase (C8), leucine arylamidase acid phosphatase, phosphoamidase, and *N*-acetyl-β-glucosaminidase were consistently strongly positive (Table 5). Thus, these reactions may be useful for identifying *A. hydrophila*.

Eight species of vibrio, including *Vibrio alginolyticus*, *V. anguillarum*, *V. carchariae*, *V. cholerae*, *V. damsela*,

V. ordalii, *V. salmonicida*, and *V. vulnificus*, have been described as causative agents of vibriosis (Egidius et al. 1986; Austin and Austin 1987). Grisez et al. (1991) reported that *V. anguillarum* could be classified into six phenotypes using API 20E. *Vibrio anguillarum* strains isolated from coho salmon (*Oncorhynchus kisutch*) were examined by API ZYM and seven profiles resulted (Table 4). These strains showed different patterns of carbohydrate fermentation (see Kurose et al. 1989). In this study, we studied only the API ZYM profiles for *V. anguillarum*, but other important vibrio species (*V. ordalii* and *V. salmonicida*) should be examined.

Pasteurella piscicida, the causative agent of pasteurellosis, and *E. tarda*, which causes edwardsiellosis, were shown to have similar enzymatic activities as assessed by API ZYM (Table 5). The biochemical characteristics of *P. piscicida* or *E. tarda* are very stable, and no biotypes have been reported.

The enzymatic activities of other fish pathogenic bacteria such as *P. fluorescens*, *F. columnaris*, and *F. maritimus* were also investigated by API ZYM in this study. The specific API ZYM profiles for each bacterial species are shown in Table 5. Only one strain of each of these species was examined, so results should be considered preliminary.

In this study, the enzymatic activities of 11 pathogenic fish bacteria were examined with the API ZYM kit in conjunction with identification using Gram stain and cytochrome oxidase and catalase assays. Moreover, the enzymatic activities and the additional tests of each bacteria were number coded for easy analysis. These code numbers identify individual bacterial species. Although other kits, including API 20NE, API 20E, and API strep, can also be used to identify fish pathogens (Hashimoto 1982; Kent 1982; Grisez et al. 1991; Iida et al. 1991; Romalde and Toranzo 1991), the application of these kits is limited to closely related species (e.g., Enterobacteriaceae, *Streptococcus*); it cannot be used for all bacterial species. We have demonstrated that the API ZYM kit can be used for almost all bacterial fish pathogens. Thus, the API ZYM test may be one of the most valuable diagnostic methods for identifying bacterial fish disease. Further studies will need to test other bacterial species such as *V. ordalii* and *Y. ruckeri*.

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Estimation of Algal Succession in Lake Water Using HPLC Analysis of Pigments

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Soma, Y., T. Imaizumi, K. Yagi, and S. Kasuga. 1993. Estimation of algal succession in lake water using HPLC analysis of pigments. Can. J. Fish. Aquat. Sci. 50: 1142–1146.

Seasonal variation in algal biomass in lake water was estimated using HPLC analysis of pigments. Carotenoids/chlorophyll *a* ratios were determined for cultures of *Anabena*, *Microcystis*, green algae, diatoms, and *Cryptomonas*. The contributions of various algal taxa to the total chlorophyll *a* content of lake water were calculated using the average carotenoid/chlorophyll *a* ratios of fingerprint pigments. The pigment analysis reflected the observed trend in the numbers of algae in lake water and proved to be a useful supplementary approach to evaluate algal biomass.

Nous avons estimé la variation saisonnière de la biomasse algale dans l'eau d'un lac en analysant les pigments par CLHP. Les rapports caroténoïdes/chlorophylle *a* ont été mesurés pour des cultures d'*Anabena*, de *Microcystis*, d'algues vertes, de diatomées et de *Cryptomonas*. La contribution des divers taxons à la concentration totale de chlorophylle *a* de l'eau ont été calculés à partir du rapport moyen caroténoïdes/chlorophylle *a* des pigments indicateurs. L'analyse des pigments reflétait la tendance observée du nombre d'algues dans l'eau du lac, et s'est révélée une approche supplémentaire utile pour évaluer la biomasse algale.

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The development of high-performance liquid chromatography (HPLC) has made it possible to completely separate and analyze the distribution of carotenoids (Züllig 1981; Mantoura and Lleywellin 1983; Wright and Shearer 1984; Goodwin and Britton 1988). Various algae have different characteristic carotenoids, in contrast with higher plants which all contain the same major carotenoids. Pigments have been increasingly used as a "biomarker" for phytoplankton in the eutrophic zone of the sea but are less studied in freshwater. Depth profiles and daily observations of carotenoids have been reported from marine systems (Gieskes and Kraay 1986a, 1986b; Strom and Welschmeyer 1991). In contrast, freshwater research has usually examined pigment degradation and preservation in lake sediments to compare the distributions of contributing organisms (Hurley and Armstrong 1990, 1991; Leavitt and Carpenter 1990; Yacobi et al. 1991).

Analysis of pigments can be an easy and useful supplementary approach to evaluate algal biomass (Gieskes and Kraay 1986b; Strom and Welschmeyer 1991), even though light and nutrients in the growth medium affect the content of light-protective algal carotenoids (Fiksdahl et al. 1983; Paerl et al. 1983). Unfortunately, algal biomass is rarely compared with pigment standing crops in lakes (Konopka and Brock 1978; Hurley and Armstrong 1990).

This paper presents the results of a study of the annual variation in algal pigments in Lake Kasumigaura and Lake Teganuma, two shallow eutrophic lakes in the central part of Japan. Extensive *Microcystis* and *Anabena* blooms develop in both lakes every summer (Goda 1984; Takamura 1984; Ogawa 1989). We estimated contributions of various algal species or classes to the chlorophyll *a* content of water using HPLC analysis of algal pigments. Average pigment composition ratios (carotenoid/chlorophyll *a*) were determined from cultured algae. Pigment-based estimates of algal abundance were compared with the composition of the phytoplankton community determined by direct microscopic counts.

Materials and Methods

Cultured strains of representative algal species found in Lake Kasumigaura and Lake Teganuma were obtained from the NIES culture collection. Cultures were maintained under a 12 h light : 12 h dark cycle at 20–27°C under standard NIES culture collection conditions (Watanabe et al. 1988). Freshwater algal species included blue-green algae (*Anabena flos-aquae* (No. 73), *Microcystis aeruginosa* (Nos. 44 and 88), *Microcystis wesenbergii* (Nos. 104 and 111), and *Aphanizomenon flos-aquae* (No. 81)), green algae (*Eudorina elegans* (No. 351), *Closterium aciculare* (No. 260), and *Tetraedron incus* (No. 392)) diatoms (*Melosira granulata* (Nos. 332 and 333) and *Synedra ulna* (No. 370)), Chrysophyceae (*Synura peterusenii* (No. 233) and *Synura spinosa* (No. 234)), and Cryptophyceae (*Cryptomonas ovata* (No. 275)). The cultures were harvested after 2–4 wk. The compositions of carotenoids and chlorophylls were analyzed using HPLC.

Water samples from Lake Kasumigaura were collected at 1 m depth every week from May 1989 to April 1991. Water samples from Lake Teganuma were collected from the lake surface once a month from March 1990 to March 1991. Aliquots of lake water were filtered through Whatmann GF/C filters (47 mmϕ) on the sampling day. Each filter was immediately frozen in liquid nitrogen and stored at −80°C until analysis. The pigment extraction and purification methods developed by Wright and Shearer (1984) were used with some modifications.

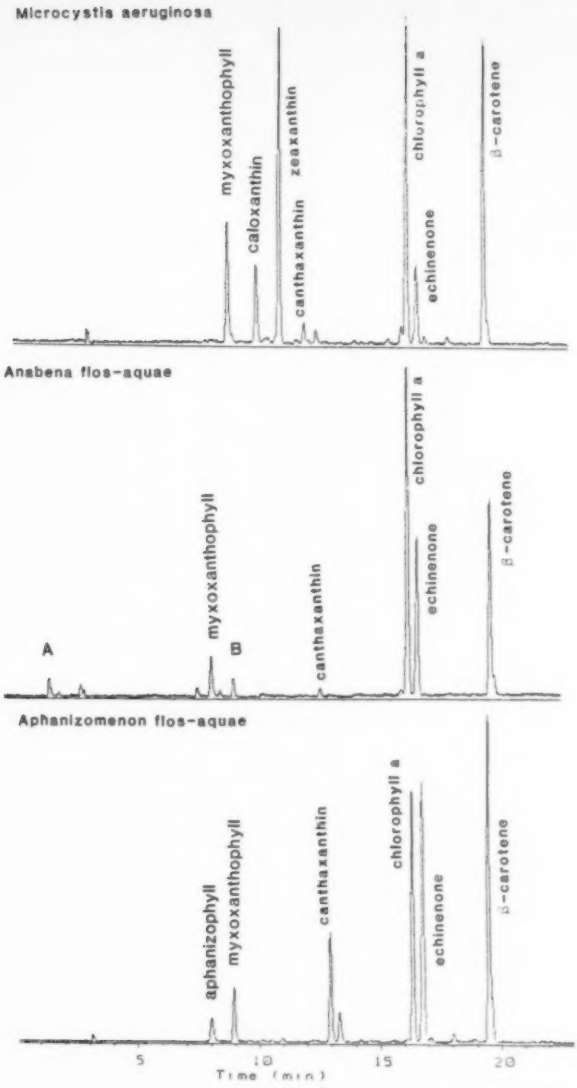

FIG. 1. Liquid chromatograms of pigment extracts from cultured strains of blue-green algae. Detection at 440 nm absorbance.

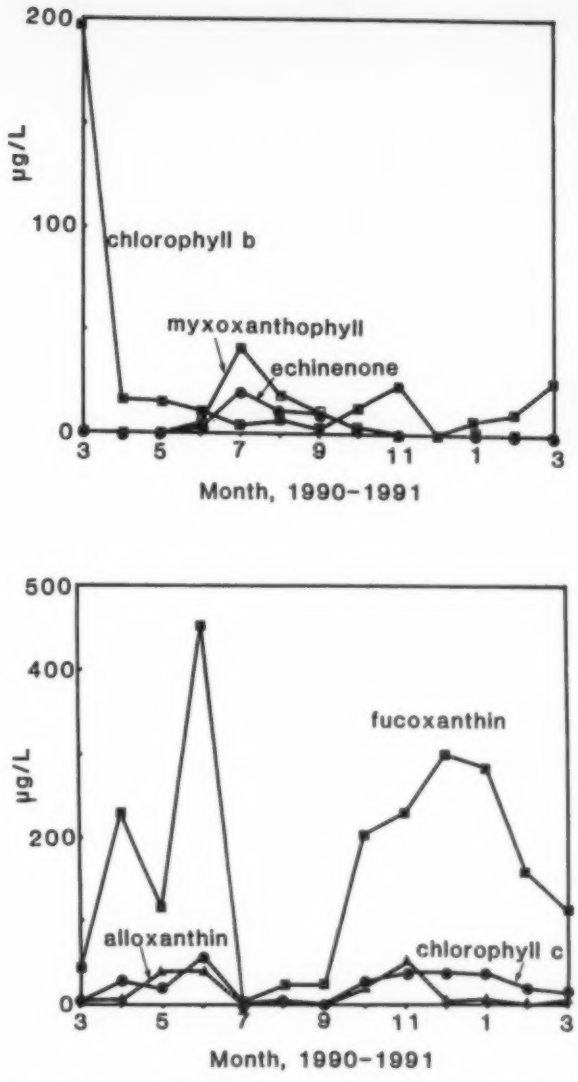

FIG. 2. Seasonal variation in pigment concentrations (µg/L water) in algae from Lake Teganuma (note the different scales on the y-axes).

The filter was sonicated for 5 min at 0°C in 5 mL of 1:1 acetone–methanol to extract the pigments immediately before the analysis. The extract was filtered through a 0.5-µm pore size Millipore FH column-guard for HPLC analysis.

The number of algal cells in water samples from Lake Teganuma was determined by direct microscopic observation, after the samples were fixed in formaldehyde.

Liquid Chromatography

The liquid chromatograph system consisted of two HPLC programmable gradient elution pumps (GL Science Co. model 576), a Rheodyne model 7125 injector, an HP (Hewlett Packard) 1040 diode array detector, a Hitachi 650-10S fluorescence spectrophotometer, and an HP 1050 workstation. Nucleosil C18 or Develosil C18 columns were used.

The separation scheme was modified from Wright and Shearer (1984) and Goodwin and Britton (1988). Mobile-phase solvent A consisted of 63:7:30 (by volume) acetonitrile – 0.5% triethylamine aqueous solution – methanol mixture, while solvent B was pure ethylacetate. Linear gradient elution from 100% A to 100% B in 20 min was followed by an isocratic hold for 5 min at 100% B. The flow rate was 1 mL/min throughout the separation. The absorption spectra from 300 to 600 nm for each of the separated pigments were obtained online with an HP 1040 diode array detector. The fluorescence detector was connected to the diode array detector to identify chlorophylls and their decomposition products. Excitation wavelength was 440 nm and emission wavelength was 660 nm.

The pigments were identified both by comparison of retention times with those of algal cultures of known pigment composition and by comparison with the absorption spectra of pure

TABLE 1. Pigment/chlorophyll *a* ratios in algae. Underscored data indicate the pigment used to determine the amount of chlorophyll *a* in algae of lake water samples.

	Anabena[a]	*Microcystis*[b]	Green algae[c]	Diatoms[d]	*Synura*	*Cryptomonas*
A	0.046					
Chlorophyll *c*				0.104	0.015	0.089
Chlorophyllide *a*				0.11		
Fucoxanthin				0.77	0.82	
Neoxanthin			0.021			
Aphanizophyll	0.028	0.012				
Violaxanthin			0.038		0.11	
Myxoxanthophyll	0.099	0.086				
Diadinoxanthin		0.05		0.39		
Anthraxanthin			0.038		0.078	
Alloxanthin						0.477
Caloxanthin?		0.107				
Diatoxanthin				0.04		
Lutein			0.20			
Zeaxanthin		0.235			0.017	
Canthaxathin	0.02	0.029	0.005			
Chlorophyll *b*			0.321			
Crocoxanthin						0.064
Chlorophyll *a*	1	1	1	1	1	1
Echinenone	0.26	0.094				
β-Carotene	0.27	0.255	0.034	0.003	0.024	0.007

[a]*Anabena flos-aquae*.
[b]*Microcystis aeruginosa*.
[c]*Eudorina*, *Closterium*, and *Tetraedron*.
[d]Melosira and Synedra.

pigments (Wright and Shearer 1984; Britton 1985). The retention time of echinenone overlapped with that of pheophytin *b*, and the retention time of β-carotene was near that of pheophytin *a2* (Vernet and Lorenzen 1987) in our separation system. The amounts of pheophytin *a1*, *a2*, and *b* were small in cultured strain samples and could be neglected.

Chlorophyll *a*, chlorophyll *b*, α-carotene, and β-carotene standards were obtained from Wako Chemical Co. Pigment quantities were determined from the peak area of the liquid chromatogram detected at 440 nm absorbance. Published specific extinction coefficients were used to determine the amounts of carotenoids (Mantoura and Lleywellin 1983; Britton 1985). The solvent effect for the specific coefficients at the detection wavelength due to the absorption peak shift was taken into consideration using on-line absorption spectra obtained with HPLC.

Methanol, acetonitrile, and ethylacetate were HPLC-grade reagents (Wako Chemical Co.) and were used after filtration and degassing without further purification.

Results and Discussion

Distribution of Carotenoids in Various Algae

Chromatograms of pigment extracts from three species of blue-green algae are shown in Fig. 1. Carotenoid glycosides such as myxoxanthophyll and echinenone are common and characteristic carotenoids contained in many blue-green algae, i.e. *Anabena*, *Microcystis*, and *Aphanizomenon* (Goodwin 1980; and see Fig. 1). Peaks A and B in Fig. 1 denote glycosides that are characteristic of some blue-green algae (Goodwin 1980; Goodwin and Britton 1988). These absorption maxima were at wavelengths similar to those of myxoxanthophyll and aphanizophyll (absorption maxima at 449, 476, and 508 nm for myxoxanthophyll; 448, 477, and 508 nm for aphanizophyll; 448, 474, and 505 nm for peak A; and 472, 503, and 544 nm for peak B, in the mobile-phase solvent). 4-Keto-myxol-2′-methylpentoside and oscillaxanthin might correspond to peaks A and B, respectively (Goodwin 1980; Züllig 1982). Further, a large amount of β-carotene (37% of carotenoids in *Anabena* and 29% in *Microcystis*) is also characteristic of blue-green algae.

The carotenoid/chlorophyll *a* ratio in each cultured strain was calculated from an average of several cultures. Mean mass ratios of pigments for *Eudorina*, *Closterium*, and *Tetraedron* were further averaged to obtain one ratio for green algae. The mass ratios of pigments from *Melosira* and *Synedra* were averaged to produce a value for diatoms. Table 1 shows the average mass ratios of pigments used to estimate the contribution of various algae to the total amount of chlorophyll *a* in lake samples. Variation in the myxoxanthophyll/chlorophyll *a* ratio was about 40% and was larger than variations in echinenone and zeaxanthin for *Microcystis* strains cultured at 25°C under a light intensity of either 1500 or 2500 lx. Burkhill et al. (1987) obtained similar pigment/chlorophyll *a* ratios based on a literature survey of green algae, diatoms, and Cryptophyceae.

Seasonal Variation in Pigments in Algae from Lake Teganuma

Seasonal variations in pigment concentrations in Lake Teganuma reveal maximum concentrations of myxoxanthophyll and echinenone in surface water during July (Fig. 2). Fucoxanthin was highly correlated with chlorophyll *c* throughout the sampling period.

The contribution of algal taxa to total chlorophyll *a* content was calculated (Fig. 3A), using the concentrations of fingerprint pigments in Fig. 2 and the pigment/chlorophyll *a* ratio from Table 1. Chlorophyll *a* calculated from carotenoids

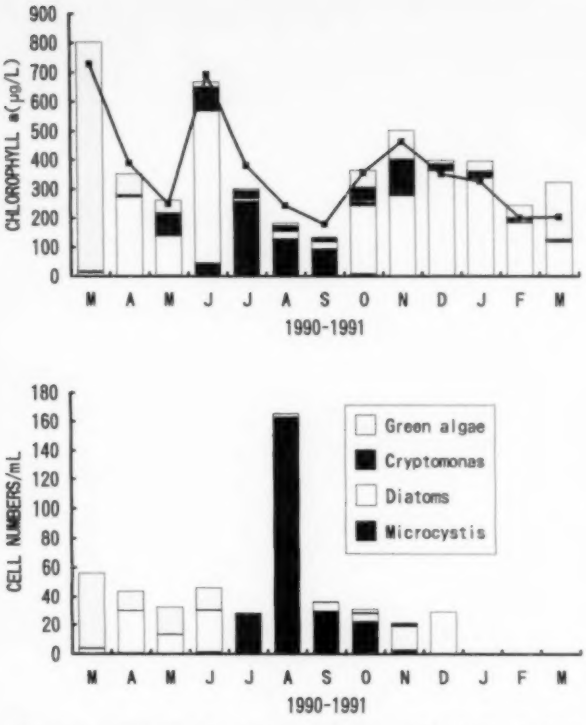

FIG. 3.(A) Contribution of various algae to chlorophyll *a* in Lake Teganuma, March 1990 to March 1991. The line shows observed chlorophyll *a* content. (B) Cell numbers of various algae in Lake Teganuma, March to December 1990.

explained the observed chlorophyll *a* within 25%, except for the March sample when green algae were dominant.

Comparison of both measures of chlorophyll with actual algal cell numbers determined by direct counts (Fig. 3B) shows that pigments were good predictors of total algal abundance except in August. Correlation factors between calculated amounts of chlorophyll *a* and direct counts of cell numbers for green algae, diatoms, and *Microcystis* in Fig. 3 (sample size = 10) were 0.91, 0.87, and 0.46, respectively. Aggregations of *Microcystis* on the lake surface made uniform sampling difficult, giving rise to discrepancies between chlorophyll *a* and direct counts during the August bloom. The correlation factor for *Microcystis* was 0.70 if the August data were omitted. Overall pigment analysis paralleled the observed trend in the number of algae in water samples from Lake Teganuma from March through December of 1990 (Fig. 3).

Blue-green algae have been reported to contain less chlorophyll *a* per cell than other algae (McCarthy and Carpenter 1979; Gieskes and Kraay 1986b). This is consistent with our observation (Fig. 3) that *Microcystis* blooms during summer do not increase the chlorophyll *a* content of lake water.

Pigment Analysis of Water Samples from Lake Kasmigaura

Pigments in the water of Lake Kasumigaura were monitored from 1989 to 1991, and the contributions of *Anabena, Microcystis*, diatoms, and other algae to the chlorophyll *a* content were calculated. Figure 4 shows the annual variation in chlorophyll *a* content calculated from the carotenoid analysis. A dense *Anabena* population developed in the beginning of the summer of 1989 but not 1990, while the population of diatoms developed through 1990.

The amount of chlorophyll *a* calculated from carotenoids in Fig. 4 was compared with observed chlorophyll *a* content in Lake Kasumigaura and is shown in Fig. 5. The average deviation throughout all seasons was less than 20%, demonstrating that the pigment analysis can reflect the contribution of algal taxa to chlorophyll *a* content in lake water and is a useful supplementary approach to evaluate algal biomass.

The calculated chlorophyll *a* contents tended to give slightly higher values than observed ones (Fig. 5). There are two possible explanations for this tendency. (1) Photosynthetic efficiencies in Lake Kasumigaura were higher than those in cultured strains. Paerl et al. (1983) indicated that increased cellular carotenoid content relative to chlorophyll *a* was accompanied by increased photosynthetic efficiency in *Microcystis*. (2) Chlorophyll may have preferentially decomposed in phytoplankton cells in lake water samples. As pheophytin *a1*, one of the decomposition products, was scarcely detected throughout all seasons, the higher photosynthetic efficiencies in the lake are the more probable cause of the higher values for calculated chlorophyll *a*.

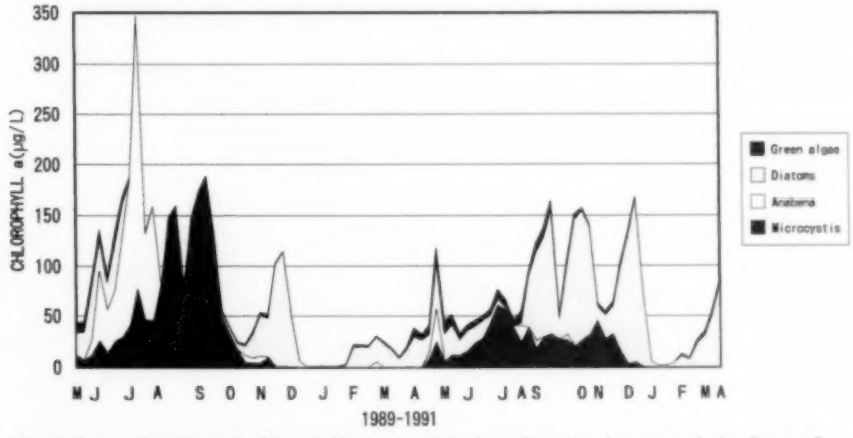

FIG. 4. Seasonal variation in chlorophyll *a* content calculated from the pigment analysis of water from Lake Kasumigaura, May 1989 to April 1991.

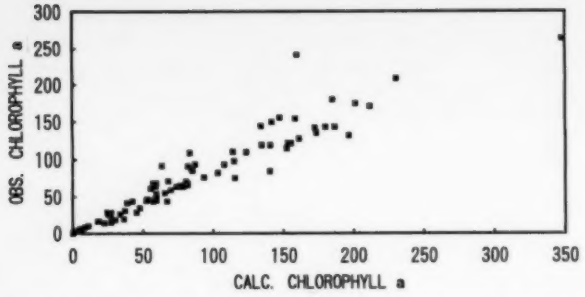

FIG. 5. Comparison of observed chlorophyll *a* content in Lake Kasumigaura with total chlorophyll *a* calculated from pigment analyses (see Fig. 4).

Concluding Remarks

Pigments were found to be good predictors of total algal abundance. Pigment composition ratios (carotenoid/chlorophyll *a*) obtained can be used to explain vertical and spatial profiles of algal taxa precisely (Gieskes and Kraay 1986a). Further, the former biomass in lakes can be estimated from pigment analysis of sediments.

Chlorophyll *a* content in Lake Teganuma during the summer did not reflect the high number of *Microcystis* cells. Pigment analysis in Lake Mendota (Hurley and Armstrong 1990) also suggests that the contribution of blue-green algae to chlorophyll *a* content is less than that of other algae.

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Genetic Variation in One Bottlenecked and Two Wild Populations of the Japanese Scallop (*Patinopecten yessoensis*): Empirical Parameter Estimates from Coding Regions of Mitochondrial DNA

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Boulding, E.G., J.D.G. Boom, and A.T. Beckenbach. 1993. Genetic variation in one bottlenecked and two wild populations of the Japanese scallop (*Patinopecten yessoensis*): empirical parameter estimates from coding regions of mitochondrial DNA. Can. J. Fish. Aquat. Sci. 50: 1147–1157.

Restriction analysis of the mitochondrial genome of 16 Japanese scallop (*Patinopecten yessoensis*) provided unique genotypes for each individual, mostly due to length variation. Fragment length variation was common, probably because tandemly repeated elements of noncoding mtDNA mutate to a different number of copies at high rates. To accurately estimate genetic diversity, we used the polymerase chain reaction (PCR) to selectively amplify coding mtDNA fragments and performed restriction analysis on these. Four pairs of PCR primers were used to amplify fragments encoding: (1) most of ATP synthetase subunit 6 and most of cytochrome c oxidase subunit 3, (2) part of cytochrome *b* apoenzyme, (3) tRNA for threonine, and (4) most of the large (16S) ribosomal gene. We used this technique to compare the genetic diversity of a population bred in a small experimental hatchery in British Columbia for three generations with its wild source population at Mutsu Bay, Aomori, Japan, and with a second wild population at Uchiura Bay, Hokkaido, Japan. The three populations were similar in the frequency distributions of the 11 mitochondrial clonal lines. This suggests that the experimental hatchery stock was not severely inbred and that gene flow between the two wild Japanese populations has been sufficient to prevent divergence.

L'analyse du génome mitochondrial au moyen d'enzymes de restriction chez 16 pectens japonais (*Patinopecten yessoensis*) a fourni des génotypes particuliers pour chaque individu, particularité due essentiellement à la variation de la longueur des fragments. Cette variation était fréquente, probablement parce que les éléments répétés en tandem de l'ADNmt non codant mutent rapidement en un nombre différent de copies. Pour estimer précisément la diversité génétique, nous avons fait appel à la réaction en chaîne de la polymérase (RCP) pour amplifier sélectivement les fragments d'ADNmt codants, puis nous avons effectué sur ces fragments l'analyse au moyen d'enzymes de restriction. Quatre paires d'amorces de la PCR ont servi à amplifier les fragments codant : 1) la plus grand partie de la sous-unité 6 de l'ATP-synthétase et de la sous-unité 3 de la cytochrome c-oxydase, 2) une partie de l'apocytochrome *b*, 3) l'ARNt pour la thréonine et 4) la plus grande partie du gène ribosomique de grande taille (16S). Nous avons employé cette technique pour comparer la diversité génétique d'une population élevée dans une petite écloserie expérimentale de Colombie-Britannique sur trois générations avec la population sauvage d'origine, qui provenait de la baie de Mutsu, région d'Aomori, au Japon, et avec une deuxième population sauvage provenant de la baie d'Uchiura, à Hokkaido, au Japon. Les trois populations étaient similaires en ce qui concerne les distributions de fréquence des 11 lignées clonales mitochondriales. Cela semble indiquer que le stock de l'écloserie ne présentait pas d'endogamie sévère, et que le flux génétique entre les deux populations sauvages japonaises est suffisant pour empêcher la divergence.

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Advances in molecular techniques have renewed interest in estimating effective population size. Populations maintained at a small effective population size for several generations can lose a substantial amount of genetic variation for quantitative traits such as growth rate and disease resistance (Lande and Barrowclough 1987; Hartl and Clark 1989). This loss is serious because the rate of response of a population to artificial selection is directly proportional to the amount of genetic variation for the traits being selected (Newkirk and Haley 1983; Falconer 1989). Small inbred populations will be severely limited in their response to either deliberate selection or selection resulting from the invasion of parasites or pathogens (Beattie et al. 1980; Lannan 1980; Newkirk 1980; Hershberger et al. 1984; Beattie et al. 1987).

Artificial rearing of bivalve larvae in commercial hatcheries can lead to small effective population sizes. Commercial hatcheries are a recent development (Hidu 1971; Lipovsky 1984), yet the North American bivalve aquaculture industry already obtains much of its bivalve seed from hatcheries (Chew 1990). To ensure that an adequate number of larvae are produced, broodstock are typically spawned in groups, often with an unequal number of males and females (e.g., Bourne et al. 1989). Group spawning can result in tremendous variation in the number of larvae produced per female; a single female bivalve can spawn millions of eggs, but if the eggs are unripe or if rearing conditions are poor, no viable larvae will be produced

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(Beaumont 1986; R. Saunders, Island Scallops Ltd., Qualicum, B.C., personal communication). The combination of using few broodstock, greatly unequal family size, and unequal sex ratios greatly increases the amount of inbreeding. Inbreeding occurs because alleles are lost when the gametes initiating the next generation are sampled from a population of finite size (Hartl and Clark 1989, p. 76). For example the effective population size of a commercial oyster stock from Humboldt Bay, California, was calculated to be only 8.9 ($\pm$2.2) individuals. This bottleneck was achieved after only three generations of hatchery propagation and was estimated from data from 14 polymorphic enzyme loci (Hedgecock and Sly 1990).

Effective population size in cultured bivalve populations has previously been estimated from nuclear genes using protein electrophoresis (Dillon and Manzi 1987; Hedgecock and Sly 1990; Vrijenhoek et al. 1990). But mitochondrial DNA (mtDNA) would be expected to be a much more sensitive measure of population bottlenecks; mtDNA is haploid, has no recombination, and usually is maternally inherited (Moritz et al. 1987). When inheritance of mtDNA is strictly maternal and sexes are separate and equally frequent the effective population size for the mitochondrial genome in a given population will be only about one quarter that of the nuclear genome (Birky et al. 1989; Hartl and Clark 1989, p. 423–424). The effective population size for the mitochondrial genome in Japanese scallop (*Patinopecten yessoensis*) populations will actually be more than one quarter that of the nuclear genome because individuals change sex when they are about 1 yr old (Osanai 1975).

In population studies, nucleotide substitutions in mtDNA are usually detected by using restriction enzymes to cut the entire circular molecule (Lansman et al. 1981). These mutations are usually rare, so two individuals that share a particular mutation are generally assumed to have descended from a common (usually maternal) ancestor (Hartl and Clark 1989). However, other mutations are not rare; the mitochondrial genomes of many animals contain length changes that result from unequal copy numbers of tandemly repeated elements of noncoding DNA regions (hereafter tandem repeats; Hale and Singh 1986; Moritz et al. 1987; Rand and Harrison 1989; Buroker et al. 1990; Wilkinson and Chapman 1991). These changes in copy number seem to occur at very high rates and two individuals that have the same number of copies do not necessarily share a common ancestor (Harrison et al. 1985; Moritz et al. 1987; Wilkinson and Chapman 1991). Thus, to correctly enumerate restriction site losses and gains, changes in fragment sizes caused by point mutations at recognition sites must be distinguished from changes in fragment sizes caused by variation in the number of copies of a tandem repeat. If the length variation caused by tandem repeats is localized to one small region, restriction sites can still be accurately located and compared; however, length variation that is present in several regions of the genome can make identification of restriction site changes difficult or impossible (Moritz et al. 1987; Bentzen et al. 1988; Mulligan and Chapman 1989). The problem is even more serious for enzymes with restriction sites within the tandem repeat, particularly if some of the repeats are incomplete (Moritz et al. 1987).

Most species of scallops are known to have extensive, dispersed size variation in their mitochondrial genome (Snyder et al. 1987; LaRoche et al. 1990; Gjetvag et al. 1992). The length variation in the sea scallop (*Placopecten magellanicus*) is known to arise from varying copy number of large (1.4 kb) tandem repeats (LaRoche et al. 1990). Attempts to use restriction analysis of the entire sea scallop mitochondrial genome as a sensitive measure of stock discrimination have been unsuccessful (Snyder et al. 1987), presumably because of difficulties in distinguishing restriction site gains and losses from size variation caused by changes in copy number of the tandem repeats and errors caused by including restriction site gains and losses created when incomplete copies of the repeats are created by mutation.

To obtain unambiguous restriction site data from the mitochondrial genome of the Japanese scallop which contained extensive, dispersed, length variation, we used four pairs of primers to selectively amplify coding regions using the polymerase chain reaction (PCR; see Mullis et al. 1986; Innis et al. 1988; Mullis 1990). We then used restriction enzymes to assess sequence variation in the amplified fragments (Hall and Smith 1991). We amplified only fragments containing coding mtDNA regions and avoided fragments with length variation, enabling us to interpret our data in terms of restriction site losses and gains. This method has the additional advantage that it can be used on very small tissue samples.

We compare the distribution of mitochondrial clonal lines (= haplotypes) in three populations of Japanese scallop. One population, chosen because it may have become inbred, was imported from Japan and cultured in a small experimental hatchery in Nanaimo, B.C., for three generations (Taylor and Bunting 1988; Bourne et al. 1989; Bourne 1991). We could not calculate the effective size of this population from the hatchery records because it is not known what proportion of the approximately five females and approximately three males that successfully spawned during each session gave rise to the larvae that survived or how many of the 10–15 spawning sessions per year provided brood stock that contributed to the next generation (B. Kingsett, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., personal communication). We compared this experimental hatchery population with its wild source population in Japan and with individuals from a second wild Japanese population.

Material and Methods

Collection Sites

Live Japanese scallop and frozen adductor muscles were obtained from Island Scallops Ltd. in Qualicum, B.C. The scallop were collected from longlines in Uchiura Bay (= Funka Bay), Hokkaido, Japan (42°N, 141°E), in December 1990 and from bottom cultured areas of Mutsu Bay, Aomori, Japan (40°50′N, 140°46′E), in April 1990. We also used scallop from the third generation of animals raised in an experimental hatchery population established at the Pacific Biological Station (Taylor and Bunting 1988; Bourne et al. 1989; Bourne and Hodgson 1991) at Nanaimo, B.C. (49°N, 123°W), from broodstock imported from Mutsu Bay between 1983 and 1986 (Bourne et al. 1989).

Isolation of Scallop mtDNA

mtDNA was extracted using a protocol developed by Snyder et al. (1987). The adductor muscle from each scallop was homogenized in a calcium-free isotonic buffer. The homogenate was incubated at 37°C for about 1 h in protease type XXIII (Sigma) to reduce its viscosity. The homogenate was then centrifuged at 39 000 *g* to pellet the mitochondria. To reduce contamination of the DNA by polysaccharides the mitochondria were resuspended in isotonic buffer, loaded onto a sucrose step

gradient, and centrifuged at 100 000 *g* at 4°C for 1 h. The band containing the mitochondria was collected from between the 1.0- and the 1.5-M layers of the sucrose step gradient. The mitochondria were pelleted again, and then lysed in a buffer containing Sarkosyl and sodium acetate. The resulting mtDNA was purified using standard procedures including several extractions with phenol/sevag (24:1, $CHCl_3$:isoamyl alcohol), an extraction with sevag, and an ethanol precipitation (Sambrook et al. 1989). The dried pellet was dissolved in 25–400 μL of TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0). For the frozen adductor muscles, this protocol was shortened; the sucrose step gradient and the spin in the ultracentrifuge were replaced by twice centrifuging the resuspended mitochondrial pellet through a layer of 1.1 M sucrose buffer at 12 000 rpm in a microfuge.

Restriction analysis of the entire mitochondrial genome was done using the protocol recommended by the manufacturers of the restriction enzymes (BRL). Eight enzymes were used: *Bam*HI, *Eco*RI, *Hin*dIII, *Hpa*I, *Pvu*II, *Sac*I, *Xba*I, and *Xho*I. The digested DNA fragments were separated in 1% agarose gels containing 0.5 μg of ethidium bromide/mL. The gels were illuminated by a short-wave UV lightbox and photographed.

To detect the small DNA fragments the digested DNA was transferred from the agarose gels to Gene Screen® nylon membrane as described by the manufacturer's directions for the capillary blot procedure. The DNA was bound to the membrane using a UV cross-linker (Stratagene).

A probe was made by labelling uncut Japanese scallop mtDNA with ^{32}P using a Prime-it Random Primer Kit® according to the manufacturer's directions (Stratagene). The unincorporated nucleotides were removed from the probe solution using a Sephadex G-25 spin column. An equal volume of formamide was added to the probe solution and the resulting solution boiled for 5 min. The solution was then kept on ice until it was added to the hybridization solution.

Hybridization and washing were done under medium stringency conditions (see Sambrook et al. 1989). Prehybridization was done in a buffer consisting of 5× SSPE, 5× Denhardts, and 0.3% SDS at 65°C for 2 h. Hybridization was done overnight in the same buffer at 65°C. Two 1-h washes were done in a buffer of 0.5× SSPE and 0.3% SDS at 65°C. Autoradiography was done at room temperature using Kodak XK1 film.

Development of Scallop mtDNA PCR Primers

mtDNA from an individual scallop was purified as described above, digested with *Eco*RI, and ligated into pUC19 plasmids (BRL) using standard procedures (Sambrook et al. 1989). The plasmids were then used to transform DH5α® competent cells (subcloning efficiency; BRL). This strain of cells was chosen because they have the mutation recA1 which means they are unlikely to lose repetitive DNA sequences. Plasmid DNA from the resulting clones was purified using Miniprep Kit Plus® (Pharmacia). Plasmids containing inserts of the same size as the bands in the *Eco*R1 digest of the original scallop mtDNA were further purified using Sephacryl® S-400 spun columns (Pharmacia). The ends of the inserts were sequenced with the Sequenase® Version 2.0 DNA sequencing kit (United States Biochemical) using the protocol described by the manufacturer and M13 universal forward and reverse primers.

Sequences were translated into all possible reading frames using the *Drosophila yakuba* mitochondrial code (Clary and Wolstenholme 1985) and the ESEE multiple sequence editor (Cabot and Beckenbach 1989). These amino acid sequences were compared with published mtDNA sequences using LFASTA (Pearson 1990). At the time, August 1991, the complete sequence of the mitochondrial genome was published for four mammals, one bird, one amphibian, one sea urchin, and one insect (Desjardins and Morais 1990). All of these groups diverged from the Bivalvia before or during the Cambrian, so identification of the poorly conserved mitochondrial regions was difficult. Nevertheless, one insert (1.5 kb) was found to contain all but about the first 10 amino acids of cytochrome *c* oxidase subunit III (COIII) and all but the last 32 amino acids of ATP synthetase subunit 6 (ATPase6), A second insert (1.4 kb) contained the last 98 amino acids of cytochrome *b* apoenzyme (Cytb) and an unidentified reading frame. A third insert (1.1 kb) contained the transfer RNA for threonine ($tRNA^{thr}$) as well as an unidentified reading frame. The identity of these three inserts has since been confirmed with the publication in June 1992 of the DNA sequence of much of the mitochondrial genome of another bivalve, *Mytilus edulis* (Hoffmann et al. 1992).

E.G. Boulding designed three sets of PCR primers (see Table 2) from the DNA sequence of the ends of these three inserts to amplify the corresponding three mitochondrial regions (hereafter COIII, Cytb, and $tRNA^{thr}$). A fourth mitochondrial region, a 0.6-kb portion of the large ribosomal subunit (hereafter 16S), was also amplified using the primers 16sar and 16sbr (Palumbi et al. 1991). The primers were synthesized at the Institute of Molecular Biology and Biochemistry at Simon Fraser University.

The PCR was performed as described by Innis et al. (1988). We used 4 μL of a dilute DNA solution as the template for the PCR. The dilute solution was made by putting 1 μL of the concentrated mtDNA solution in TE into 1 mL of sterile double-distilled water. The PCR machine (Ericomp®) was set for 35 cycles with an annealing temperature of 48°C for 30 s for all pairs of primers. After the PCR cycles were completed the solution containing the amplified mtDNA fragments was carefully removed from under the mineral oil so that no oil was transferred to the clean tube.

The restriction digests consisted of 4 μL of the PCR mixture, 6.5 μL of sterile distilled water, 1 μL of the appropriate 10× buffer, and 0.5 μL of the restriction enzyme. Restriction digests were incubated for at least 2 h, but digests with *Hae*III benefitted from being left for 4 h. Reactions were stopped using 1 μL of 50% glycerol, 0.25% bromphenol blue, and 0.25% xylene cyanole and then heated at 65°C before loading. The digests were loaded onto 1% agarose gels containing 0.5 μg of ethidium bromide/mL. The gels were run under standard conditions and then photographed under UV light.

Twelve restriction enzymes (see Table 2) were screened for each of the four amplified regions from each of 16 individual scallops. Enzymes not showing variation in the test individuals were not used further in the survey. Samples in which a site loss or gain was observed were reamplified to ensure that the variant was not an error made by the *Taq* polymerase. The sample was then redigested for a longer period to ensure that the loss or gain was not an artifact of a partial digest.

The 10 four-base restriction enzymes sampled 74 sites and the two six-base restriction enzymes sampled 12 sites to make a total of 306 base pairs sampled for at least 16 test individuals. Each of the four amplified fragments from each of the 52 individuals was digested with the enzymes that had shown poly-

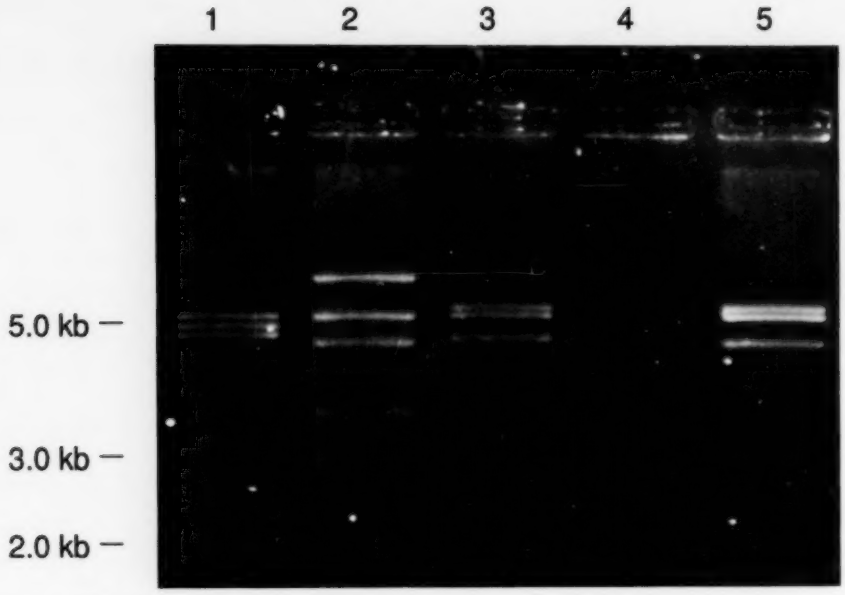

FIG. 1. Restriction digest of the entire mitochondrial genomes of four Japanese scallop with *Eco*RI visualized with ethidium bromide staining. Lane 1, H17; lane 2, H18; lane 3, H19; lane 4, blank; lane 5, H21 (Table 1). kb = kilobases.

morphism for the first 16 individuals sampled. This resulted in sampling 140 nucleotide sites (see Table 3). The photographs of the digests were easy to interpret in terms of site losses and gains because most involved only one or two variants.

All restriction sites recognized by a single restriction enzyme in all four PCR-amplified mitochondrial regions were considered to be the set of characters for that enzyme (see Tables 3 and 4); this enabled the use of the standard restriction site analysis packages REAP 1.1 (McElroy et al. 1992) and PHYLIP 3.41 (Felsenstein 1991). The program DA in REAP was used to compute the haplotype (=mitochondrial clone) diversity (Nei 1987, equations 8.5 and 8.12) and nucleotide diversity (polymorphism per nucleotide site) within and between populations (Nei 1987, equations 10.6 and 10.19–10.21). The program MONTE in REAP was used to do 5000 Monte Carlo simulations to test whether the chi-square value (χ^2) for the distribution of the mitochondrial clones in the three populations was larger than that expected by chance alone (Roff and Bentzen 1989). Finally, the program RESTML in PHYLIP 3.41 was used to estimate a phylogeny of the mitochondrial clones based on the restriction site characters; this program uses a maximum likelihood algorithm (Felsenstein 1992).

Results

Restriction Analysis of Entire Mitochondrial Genome

Figure 1 shows that not all restriction patterns from digests of the entire mitochondrial genome of the Japanese scallop can be interpreted in terms of restriction site losses and gains. Individuals H17, H19, and H21 had the same number of bands when digested with *Eco*RI, but the second largest band in the digest of H17 is the largest band in the digest of H19 and H21. Since the number of fragments was unchanged, this increase in the size of the band was likely caused by an increase in the number of copies of a tandem repeat. All the other seven restriction enzymes also revealed extensive size variation for at least one of the fragments that could not be explained in terms of the loss or gain of a restriction site (E.G. Boulding, unpublished data).

Table 1 illustrates the complications that arise if size variation caused by the gain and loss of restriction sites is not separated from size variation caused by changes in the number of copies of a tandem repeat. For example the size variation for the *Hin*dIII digest was confined to the largest fragment. Six sizes of this fragment were observed. We designated these as genotypes a–f. If a similar procedure is used to assign genotypes to the restriction patterns from *Bam*HI and from *Eco*RI, then each of the 16 scallops can be assigned a unique composite genotype based on just these three enzymes.

Unfortunately the genotypes arising from size variation caused by changes in the number of copies of tandem repeats do not seem to be useful for estimating population genetic parameters. There was no trend for certain size variants to be population specific. There was also no trend for the frequency of a given size-class of fragment to be different in the different populations (Table 1).

There was also no relationship between the total size of the mitochondrial genome and the population from which the individuals were sampled (Table 1). The size of the entire mtDNA molecule of the Japanese scallop ranged from 27 to 32 kb for different individuals (Table 1).

Useful population genetic parameters could probably be obtained by assigning different genotypes only to variants resulting from site losses or site gains, but the extensive dispersed size variation made it difficult to score these separately. Whereas genotype c of *Bam*HI has obviously lost a restriction site that genotype d possesses (Table 1), it is less clear how many sites have been lost between genotypes a and

TABLE 1. Restriction digests of the entire mitochondrial genome of Japanese scallop from Hokkaido (H), Aomori (A), and Nanaimo (N). Approximate band sizes are given in kilobases (kb) so that different individuals can be compared. The different restriction patterns for eachenzyme have been assigned different "genotypes" (a–m) for purposes of discussion. Total genome sizes are approximate and were measured in digests with other enzymes in which all bands were smaller than 5 kb.

H17	H18	H19	H21	H23	H24	A28	A34	A35	A36	N29	N30	N31	N33
					*Hin*dIII								
a	b	c	?	c	d	d	a	a	d	e	f	b	a
7.7	8.7	8.4	8.4		9.6	9.6	7.7	7.7	9.6	8.1	6.3	8.8	7.7
5.6	5.6	5.6	5.6		5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6
5.4	5.4	5.4	5.4		5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4
											3.4		
2.9	2.9	2.9	2.9		2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9
0.2	0.2	0.2	0.2		0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
0.1	0.1	0.1	0.1		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
					*Bam*HI								
a	b	c	d	e	f	?	g	h	i	j	k	l	m
	9.6	11.9							11.6				12.3
8.6	8.6	8.6	8.6	8.6	8.6	8.6		8.6	8.6	8.6	8.6	8.6	8.6
7.7			6.8	7.2	7.2	7.2		6.5		6.0	7.7	7.0	
5.5	5.5	5.5	5.5	5.5	5.5	5.5		5.5	5.5	5.5	5.5	5.5	5.5
3.7	3.4		3.4	3.7	3.4	3.4		3.5		3.3	3.1	3.2	
					*Eco*RI								
a	b	c	c	d	d	d	d	d	d	d	e	d	f
	6.7	6.1	6.1										
5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4
5.3				5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3
5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
										4.0			
	3.8									3.8			
	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3
2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9
2.3		2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3		2.3	2.3	2.3
1.6													1.6
1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
1.3		1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3		1.3	1.3	1.3
1.3		1.3	1.3	1.3								1.3	1.3
1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1		1.1
					Approximate genome sizes								
27.9	29.6	30.4	30.4	28.3	28.3	28.3	28.3	28.3	28.3	32.4	28.3	29.5	29.5

b of *Eco*RI (Fig. 1; Table 1). The situation is further complicated by the presence of heteroplasmic individuals that contain more than one type of mitochondrial genome. Therefore, standard restriction analysis of the entire mitochondrial genome of the Japanese scallop did not result in useful estimates of population genetic parameters.

Restriction Analysis of PCR-Amplified mtDNA Fragments

The four fragments amplified with PCR did not seem to contain any regions with tandem repeats. No variation in fragment size was seen except that attributable to restriction site loss and gain. This was true for all four amplified products from the four sets of primers (Table 2).

All four of the PCR products showed variation for the presence or absence of at least one restriction site (Fig. 2; Table 2). Some restriction enzymes seem to be more likely to have polymorphic sites; *Hpa*II, which recognizes CCGG, had polymorphic sites for three of the four amplified regions. Further, some mitochondrial regions showed more polymorphism per unit length than others; the fragment COIII (1.6 kb) contained five polymorphic restriction sites whereas the fragment Cytb (1.5 kb) contained only one (Table 2). A total of 10 variable restriction sites were found in the 52 individuals that were sampled (Table 3).

The 10 variable restriction sites defined 11 mitochondrial clones (Table 4). Three of these clones contained at least five individuals, while the other eight contained only one or two individuals. Of these eight rare clones, four were found only in the Hokkaido population, three were found only in the Nanaimo population, and one was found only in the Aomori population.

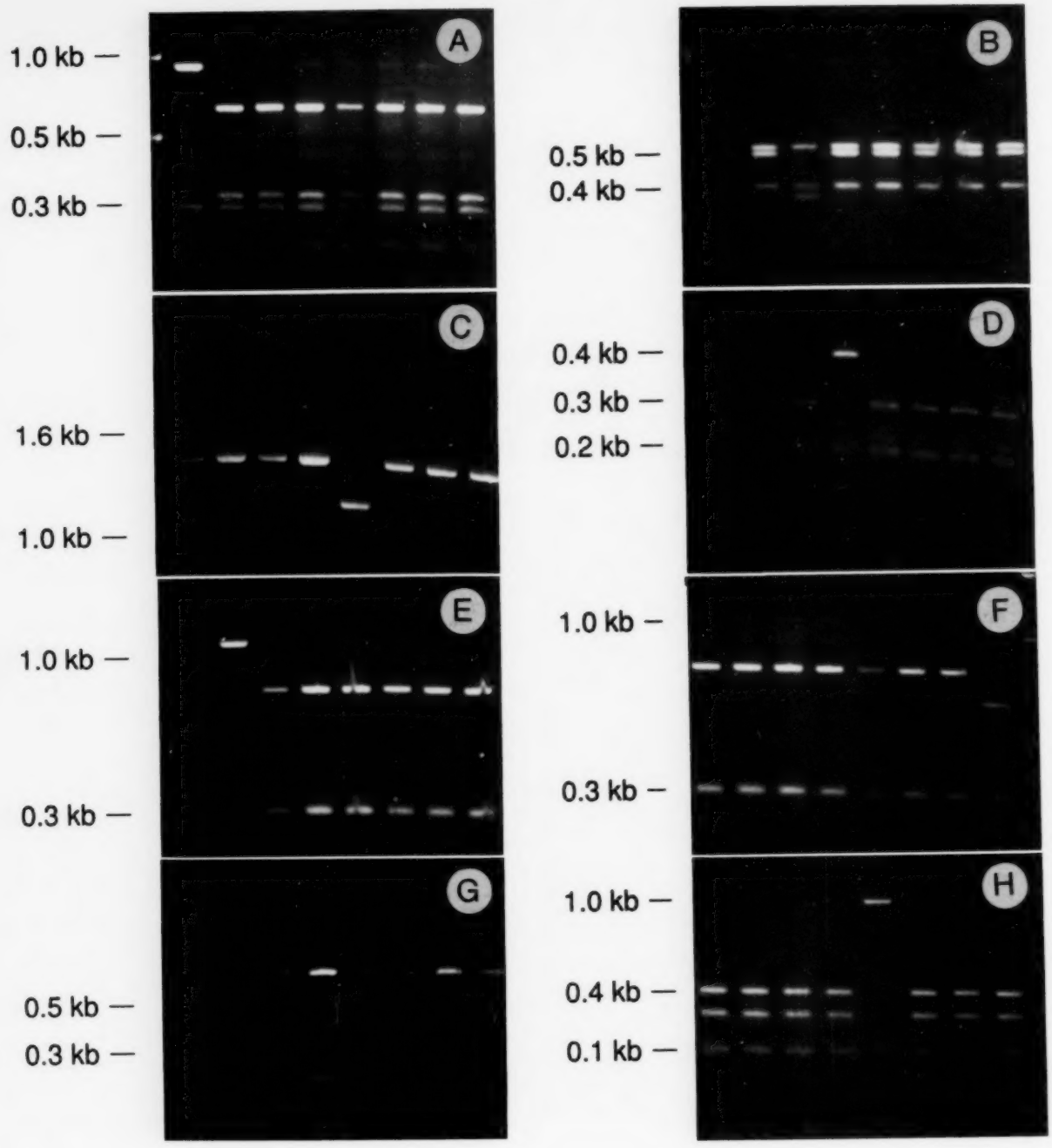

FIG. 2. Restriction digests of PCR-amplified mitochondrial fragments (see Table 2) showing restriction site mutations used as genetic markers. (A) Site a: Cytb fragment digested with *Hae*III; (B) site b: COIII fragment digested with *Hha*I; (C) site c: COIII fragment digested with *Hpa*II; (D) site d: 16S fragment digested with *Hpa*II; (E) site f: COIII digested with *Sau*96I; (F) site g: COIII digested with *Sau*96I; (G) site h: $tRNA^{thr}$ digested with *Sau*96I; (H) site j: COIII digested with *Taq*I. The smallest detectable fragments (between 0.05 and 0.2 kb) are not distinct in these photographs but were observed in the negatives. Two genetic markers, site e ($tRNA^{thr}$ digested with *Hpa*II) and site i (tRNA fragment digested with *Sau*96I), are not shown. kb = kilobases.

TABLE 2. PCR primers used to amplify mtDNA fragments from Japanese scallop. The fragments were then digested with four-cutter restriction enzymes. Primer sequences are given 5′ to 3′.

Primer name	Fragment size (kb)	Fragment identity	Enzymes[a] showing variation
Clone 40F; 40R[b] agggctctatccgctaggca cgcctccccgataatagcc	1.5	Most of ATPase6 and nearly all of COIII	*Hha*I, *Hpa*II, *Sau*96I (2 sites), *Taq*I
Clone 41F; 41R[b] cgtgtgcttgcccaggtagg aattctccgctctattccga	1.4	End of cytochrome *b*	*Hae*III
Clone 42F; 42R[b] caggtggtttggggtgtggg cccggctcaacacctgacag	1.1	?; $tRNA^{thr}$	*Hpa*II, *Sau*96I
16 sar; 16sbr[c] cgcctgtttatcaaaaacat ccggtctgaactcagatcacgt	0.6	16S ribosomal gene	*Hpa*II

[a]Restriction enzymes screened included *Alu*I, *Dde*I, *Dra*I, *Hae*III, *Hha*I, *Hin*fI, *Hinc*II, *Hpa*II, *Mbo*I, *Rsa*I, *Sau*96I, and *Taq*I.

[b]Primers designed by E.G. Boulding from cloned *Patinopecten yessoensis* mtDNA sequence.

[c]Universal primers from "The Simple Fool's Guide to PCR" ver. 2 by S. Palumbi et al., University of Hawaii at Honolulu.

TABLE 3. Inferred restriction site profiles for all four mtDNA fragments that were amplified with PCR. The letters A–D refer to composite genotypes for each enzyme for all four fragments combined. The enzyme *Hinc*II was also used but did not cut any of the four fragments. +, site present; −, site absent; N, enzyme did not cut. The superscript letters a–i refer to the 10 variable restriction sites. See Fig. 2 for restriction patterns and fragment sizes.

	COIII	CytB	16S	$tRNA^{thr}$
*Alu*I				
A	+ + +	+ + +	+ +	N
*Dde*I				
A	+ + + +	+ + +	+ +	+ +
*Dra*I				
A	N	+	N	+
*Hae*III				
A	+ + +	+ + +	+	+ +
B	+ + +	+ + −[a]	+	+ +
*Hha*I				
A	+ − +	+	N	N
B	+ +[b] +	+	N	N
*Hin*fI				
A	+ + +	+ + +	+ +	+ +
*Hpa*II				
A	+ −	+ + +	+ +	+ −
B	+ +[c]	+ + +	+ +	+ −
C	+ −	+ + +	+ −[d]	+ −
D	+ −	+ + +	+ +	+ +[e]
*Mbo*I				
A	+ +	+ +	+	+
*Rsa*I				
A	+ + +	+ +	N	N
*Sau*96I				
A	+ + −	+	+ +	+ + − +
B	+ −[f] −	+	+ +	+ + +[h] +
C	+ + +[g]	+	+ +	+ + − +
D	+ + −	+	+ +	+ + − −[i]
*Taq*I				
A	+ + +	+ + + +	N	N
B	+ + −[j]	+ + + +	N	N

There was no significant difference in the frequencies of the 11 clones ($\chi^2 = 20.38$, df = 10) in the three populations. This was true even when all rare clones were combined into a single cell ($\chi^2 = 4.8$, df = 3). Monte Carlo simulations (Roff and Bentzen 1989) showed that the probability of exceeding these χ^2 values by chance alone was very high (0.466 ± 0.007 and 0.610 ± 0.007, respectively; $N = 5000$ replicates per simulation).

The maximum likelihood estimation of the phylogeny of the 11 clones showed only one significant clade (= branch). This clade consisted of five clones that appeared together five out of six runs of the program (Fig. 3). All of the five clones in this clade had lost restriction site a (*Hae*III, Table 3) relative to the reference genotype, clone I. No other clades were present, since all of the other five clones differed from clone I by only a single restriction site (Fig. 3).

When the geographic locations were mapped onto the phylogeny, there was no relationship between a clone's position on the phylogeny and the populations in which the clone was found (Fig. 3). The clade of five clones (Fig. 3) contained individuals from all three populations. There was no evidence for a clade of restriction site mutations that was unique to a particular population.

The mean within-population haplotype diversity was highest for the Hokkaido population and lowest for the Nanaimo population, but the differences were not significant (Table 5). The mean within-population nucleotide diversity was greatest for the Hokkaido population and lowest for the Nanaimo population. The between-population component of the nucleotide diversity was greatest between the Aomori and the Hokkaido populations and smallest between the Aomori and the Nanaimo populations (Table 5). Negative estimates of the nucleotide divergence between populations were obtained for two of the three pairwise comparisons of the three populations (Table 5), likely because this index is calculated by subtracting the within-population nucleotide divergence from the total nucleotide divergence and most of the variation was within rather than between populations.

TABLE 4. Number of individuals with each mitochondrial clone found in the three populations: Uchiura Bay, Hokkaido; Mutsu Bay, Amori, Japan (source population); and Pacific Biological Station, Nanaimo, B.C. See Table 3 for restriction patterns of the genotypes.

Clone	Genotype	Hokkaido	Amori	Nanaimo
I	AAAAAAAAAAA	7	7	14
II	AAABAAAAABA	1	0	0
III	AAAABAAAAAA	1	0	0
IV	AAABAACAAAA	1	0	0
V	AAAAAABAAAA	2	0	0
VI	AAABAAAAAAA	1	1	4
VII	AAAAAADAAAA	1	1	4
VIII	AAABAAAAAAB	0	0	1
IX	AAAAAAAAACA	0	0	1
X	AAABAAAAADA	0	1	0
XI	AAAAAAAAAAB	0	0	1

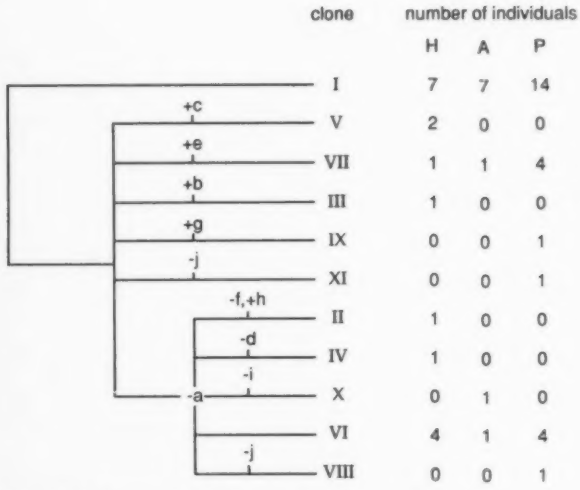

FIG. 3. Phylogeny of the mitochondrial clones estimated from restriction site data using a maximum likelihood algorithm (Felsenstein 1992). The letters a–j represent a gain (+) and a loss (−) at that site relative to the outgroup, clone I. Three Japanese scallop populations were sampled: H = Hokkaido, A = Aomori, P = Nanaimo.

TABLE 5. Nucleotide diversity within and among populations. IHJ = Hokkaido, PBS = Nanaimo, and IAJ = Aomori.

Population	Haplotype diversity (selfing) (±SE)	Nucleotide diversity
IHJ	0.79 ± 0.08	0.0021
PBS	0.53 ± 0.18	0.0012
IAJ	0.66 ± 0.09	0.0014
Mean	0.66 ± 0.01	0.0016

Nucleotide diversity (above diagonal) and nucleotide divergence (below diagonal) among populations

	IHJ	PBS	IAJ
IHJ		0.0017	0.0018
PBS	−0.000021		0.0013
IAJ	0.000023	−0.000054	

	Nucleotide diversity	Nucleotide divergence
Mean	0.0016	−0.000017
Minimum	0.0013	−0.000054
Maximum	0.0018	0.000023

Discussion

This method of assessing the frequency distribution of mtDNA clones is sensitive and would have detected severe inbreeding in the experimental hatchery population from Nanaimo had it existed. Our PCR primers amplified only coding regions of the mitochondrial genome, and our amplified regions showed no length variation and are unlikely to contain tandem repeats. Therefore, we are confident that the frequencies of the mitochondrial clones we detected can be used to obtain accurate estimates of population genetic parameters.

After only three to five generations at a small population size, new mutations are unlikely, so each of the six clones now present in the Nanaimo population must represent at least one female-acting parent that was present during the bottleneck. Indeed, considerably more than six female-acting parents must have been present, since the frequency distribution of the clones was not significantly different from its source wild population from Aomori (Table 4; Fig. 1). Simulations, using a haploid genetic algorithm suggest that the hatchery population must have been at least 15–20 individuals, assuming the bottleneck was maintained for five generations, because the rare haplotypes in the source population were not lost in the hatchery population (E.G. Boulding and T. Hay, unpublished data).

Male-acting parents also may have contributed to the observed variation in the clones if significant paternal inheritance of mtDNA occurs in Japanese scallop, but we think that this is unlikely. There is evidence of biparental inheritance of mtDNA in another bivalve, *Mytilis edulis*, but *Mytilis* may be exceptional because of extensive hybridization among three closely related species (Hoeh et al. 1991).

The rank order of haplotype diversity and the within-population nucleotide diversity was greatest in the wild population from Hokkaido and least in the experimental hatchery population from Nanaimo (Table 5). However, the differences were not significant, again suggesting that the experimental hatchery stock was not substantially inbred.

There were no clades of mitochondrial clones unique to either of the wild populations in Japan (Fig. 3), which had similar frequencies of 11 mitochondrial clones (Table 4). The frequencies could be similar because of gene flow between the two wild populations. Scallop larvae are planktonic for about

1 mo and could disperse long distances before they settle (Yamamoto 1975). The similar frequencies could also occur because the Japanese aquaculture industry collects natural scallop seed on artificial substrates and then moves the juveniles to bays all over the coast of Northern Japan (Ito et al. 1975; R. Saunders, Island Scallops Ltd., Qualicum, B.C., personal communication). Ito et al. (1975) recorded that seed has been brought into Mutsu Bay, Aomori, from Hokkaido.

Previous studies have used allozyme electrophoresis to investigate inbreeding in captive bivalve populations. Most studies of inbred populations have failed to detect a decline in average proportion of allozyme loci that are heterozygous per individual (Dillon and Manzi 1987; Hedgecock and Sly 1990; Vrijenhoek et al. 1990). This index is insensitive and can only detect extreme bottlenecks because it confounds allelic richness and evenness (Watterson 1984). McCommas and Bryant (1990) calculated that to be 80% sure of detecting even an extreme one-generation bottleneck of four pairs of houseflies at the 5% level of significance, it is necessary to assay at least 15 polymorphic loci for 30 individuals. An alternative index is the change in allele frequency between the wild source and hatchery populations in the mean number of alleles per locus. Hedgecock and Sly (1990) found a significant decrease in the mean number of alleles per locus between the source and one of two hatchery populations using 14 loci with a sample size per locus of 57.5 Pacific oyster (*Crassostrea gigas*). However, the amount of allozyme variation present in Japanese scallop populations (Nikiforov and Dolganov 1982) is known to be low relative to other bivalves (Beaumont 1986) and would therefore not be a powerful way of assessing inbreeding.

Only one previous study has used mtDNA to assess inbreeding in bivalve populations. Brown and Paynter (1991) used restriction analysis of the mitochondrial genome to compare the distribution of haplotypes of a hatchery population of the eastern oyster (*Crassostrea virginica*) with that of some wild source populations. They concluded that the hatchery population was severely inbred.

The greater sensitivity of mtDNA occurs in part because it is easily studied at the level of the nucleotide instead of that of the protein. Mutations in third codon positions of protein-coding genes do not change the amino acid and are more likely to be close to neutral with respect to fitness than are amino acid substitutions that change the net charge and thus the allelic allozyme (Hartl and Clark 1989). This means that many more potential markers should be maintained in a population (Hartl and Clark 1989). Indeed, Chapman (1989) found that mtDNA clonal lines were more sensitive than nuclear markers (allozymes) for detecting bottlenecks due to founder effects in three introduced populations of bluegill (*Lepomis macrochirus*). He reported mtDNA divergence among three populations over a 20-yr period but observed no divergence in the allozyme frequencies.

There is no simple relationship between single locus molecular markers such as allozymes or restriction site changes and most ecological traits (Lande and Barrowclough 1987) because genetic variation in many ecological traits, such as growth rate and fecundity, is caused by (quantitative) genetic variation at many loci that each have a small effect on the trait (Falconer 1989). Experimental studies where housefly populations were put through various population bottlenecks have demonstrated that single locus markers (allozymes) may lose genetic variation at a higher rate than do quantitative traits (Bryant et al. 1990; McCommas and Bryant 1990).

Not all population bottlenecks need have an adverse effect on quantitative genetic variation for ecologically important traits, particularly when the bottleneck is of short duration. Chew (1990) described how 26 individual bay scallop from the eastern United States were introduced to a hatchery in China and within 5 yr gave rise to a fishery producing 50 000 tonnes per year. A key point here is that the 26 individuals were derived from a large, wild population and gave rise to millions of descendents within a few generations. However, a bottleneck of 26 individuals would result in severe inbreeding if it were maintained for many generations (Lande and Barrowclough 1987).

This method of assaying inbreeding using restriction analysis of PCR-amplified fragments is faster and cheaper than direct sequencing of DNA (Innis et al. 1988) yet provided good resolution. Now that we know the restriction patterns for the different scallop genotypes, the processing time could be decreased by almost one quarter by combining all the PCR-amplified fragments for each scallop into one tube, digesting an aliquot from the tube with each enzyme, and then running each digest in a single lane. Our method is not technically difficult and does not require use of expensive equipment or highly trained technicians.

The efficiency of this method can also be increased by using PCR primers for fast-evolving protein-coding genes rather than for ribosomal genes. We found twice the number of restriction site polymorphisms per kilobase amplified in fragments containing protein-coding genes as in fragments containing ribosomal genes. All protein-coding regions were not equally variable; we found five restriction site polymorphisms in the ATPase6-COIII fragment and only one in the Cytb fragment. Similar trends were found (Latorre et al. 1992) in the mtDNA from old world populations of fruit flies (*Drosophila subobscura*). They found no variable restriction sites in ribosomal genes but found nine variable sites out of a total of 13 in regions coding for the NADH protein complex.

In conclusion, this method of using molecular markers in mtDNA allows estimation of the effective population size of hatchery populations of scallop relative to wild populations. mtDNA is a sensitive indicator of historical bottlenecks in such populations. Detection of a historically small effective population size that is maintained for a number of generations can be an indication of severely reduced genetic variation in ecologically important quantitative traits; thus, periodic monitoring of any bivalve hatchery stock is important because the conditions under which bivalve seed are produced can easily result in a small effective population size.

Future work on Japanese scallop could involve fingerprinting of female broodstock and larvae to see whether most of the larvae are produced by a few of the females. This might be done by making PCR primers that enable amplification of tandem repeat regions. This method of fingerprinting would probably be successful in bats. Wilkinson and Chapman (1991) used PCR and image analysis to assay the number of copies of a short tandem repeat (81 base pairs) in 58 mother bats and an average of three of their offspring. They found only one offspring that was heteroplasmic for a different number of copies of tandem repeats than its homoplasmic mother had had. If the number of copies of the larger tandem repeats in the scallop mitochondrial genome are also usually transmitted stably from mother to offspring, then PCR of these regions might enable hatcheries to determine which female-acting parents have produced viable larvae.

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Use of Path Analysis to Investigate Mercury Accumulation in Brown Trout (*Salmo trutta*) in Norway and the Influence of Environmental Factors

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Fjeld, E., and S. Rognerud. 1993. Use of path analysis to investigate mercury accumulation in brown trout (*Salmo trutta*) in Norway and the influence of environmental factors. Can. J. Fish. Aquat. Sci. 50: 1158–1167.

We studied relationships between environmental factors and mercury accumulation in resident brown trout (*Salmo trutta*) in 25 lakes in Norway. Our survey showed that mercury concentrations in fish are elevated in areas receiving high atmospheric depositions of mercury. A path analysis revealed that the concentrations of total organic carbon had a critical influence on the bioaccumulation of mercury. This reflects the role of humic matter in the transportation of mercury to lake water and sediments, and perhaps also a stimulating effect on the microbial methylation of mercury. Lake depth influenced the concentration of mercury in sediments, probably due to the complexation of mercury with sedimenting humic matter. The effect of such sediment on mercury concentrations in fish indicated that there exists a concentration-dependent production and release of methyl mercury to the biota. Selenium deposited from the atmosphere seemed to lower the bioavailability of mercury to fish. No effect of pH could be detected. The strong effect of total organic carbon implies that even moderate atmospheric deposition rates may result in relatively high mercury levels in fish, if other local environmental factors favour this.

Nous avons étudié les relations entre les facteurs environnementaux et l'accumulation de mercure chez les truites brunes (*Salmo trutta*) résidentes dans 25 lacs de Norvège. Notre étude a montré que les concentrations de mercure chez les poissons sont élevées dans les régions qui reçoivent des dépôts importants de mercure d'origine atmosphérique. Une analyse causale a révélé que les concentrations de carbone organique total avaient un effet critique sur la bioaccumulation de mercure. Ce phénomène révèle le rôle de la matière humique dans le transport du mercure vers l'eau et le sédiment des lacs, et peut-être aussi son effet stimulant sur la méthylation du mercure par les microbes. La profondeur du lac influait sur la concentration de mercure dans les sédiments, ce qui est probablement dû à la complexation du mercure avec la matière humique en voie de sédimentation. L'effet de ces sédiments sur les concentrations de mercure chez les poissons indique qu'il existe une production et une concentration du méthylmercure dans le biote qui sont dépendantes de la concentration. Le dépôt de sélénium provenant de l'atmosphère semblait réduire la biodisponibilité du mercure pour les poissons. On n'a pu détecter aucun effet du pH. L'influence importante du carbone organique total semble indiquer que même des taux modérés de dépôt atmosphérique peuvent avoir pour effet des concentrations relativement élevées de mercure chez les poissons, si d'autres facteurs environnementaux favorisent ce phénomène à l'échelle locale.

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Mercury contamination of freshwater fish is a severe problem in both Fennoscandia and North America. A large number of remote lakes contain fish with mercury concentrations that often exceed those considered safe for human consumption (0.5–1.0 $\mu g \cdot g^{-1}$) (Andersson et al. 1987; Sorensen et al. 1990; Verta 1990; Wiener and Stokes 1990). This contamination is supposed to be a result of atmospheric deposition of and anthropogenic mercury to lakes and their catchment areas; however, the relationship between the deposition rates and mercury concentrations in fish is not well understood. Our primary objectives in this study were to examine the empirical relationship between mercury in populations of brown trout (*Salmo trutta*) and the environmental characteristics of lakes in Norway and to test by statistical modelling the explanatory power of a number of proposed causal mechanisms.

The variation in mercury concentration within populations of a given species is largely controlled by the size or age of the fish. The variation between allopatric populations is suggested to be regulated by the mercury load in lakes and the bioavailability of the element (Nilsson et al. 1989; Cope et al. 1990; Verta 1990; Winfrey and Rudd 1990; Wren et al. 1991). However, a number of factors are suspected to interfere in a complex way. While atmospheric deposition is the main source of mercury to catchment areas, the supply to lakes is largely controlled by the amount of organic carrier particles in inflowing rivers (Meili 1991). Inorganic Hg(II) ions are adsorbed to humic colloids, which in turn act as vehicles for mercury to enter the aquatic ecosystem from the catchment area (Lee and Iverfeldt 1991; Mierle and Ingram 1991). Sedimentation processes affect the retention of unassimilated mercury within lakes (Johnson

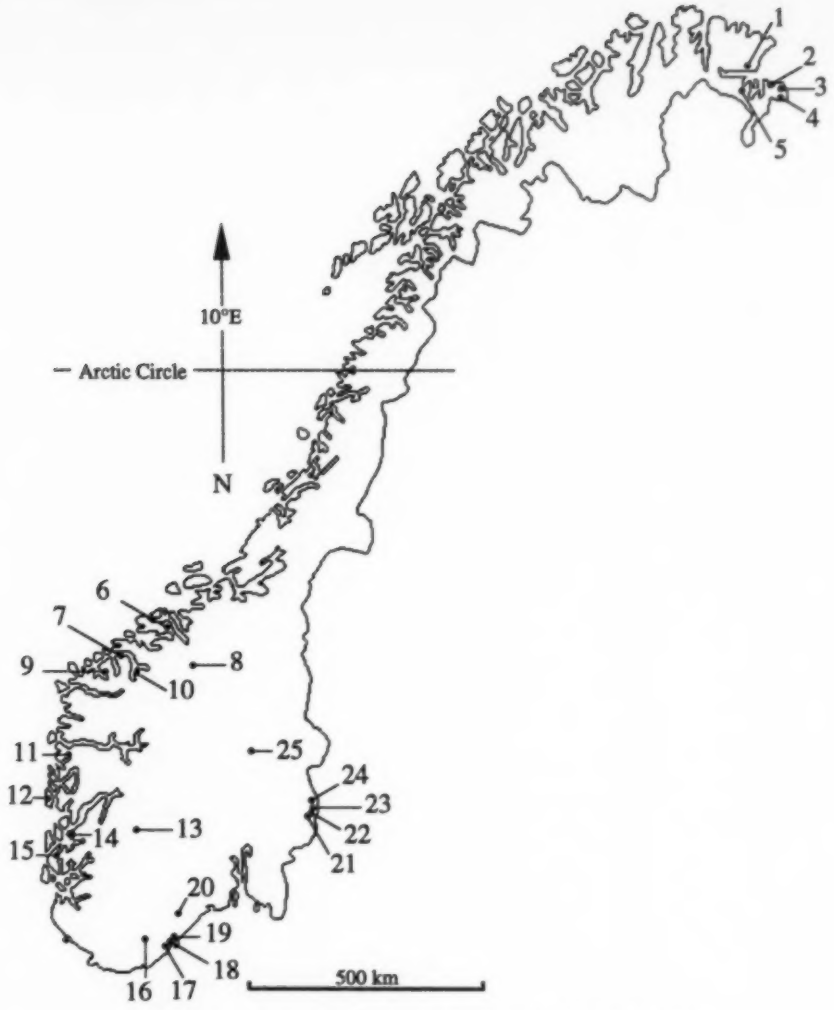

FIG. 1. Map of Norway showing the distribution of the lakes studied.

1987; Santschi 1988), with both the sediment surface and water column being important sites for the microbial methylation of mercury (Korthals and Winfrey 1987; Xun et al. 1987; Parks et al. 1989).

Methylmercury (MeHg) is an extremely toxic substance that accumulates readily in biota; most of the mercury in fish exists in this form (Westöö 1966; Grieb et al. 1990). The net methylation rate in a lake ecosystem depends on a number of environmental factors, of which the most important are thought to be microbial activity (stimulated by decomposable organic matter), mercury adsorbing onto colloids such as humic matters, clays, and hydrated metal oxides, pH, redox conditions, and divalent binding anions such as sulfide and selenide (Rudd et al. 1983; Björnberg et al. 1988; Jackson 1988, 1989; Winfrey and Rudd 1990).

Thus, the factors controlling mercury content in fish are complex, often interrelated, and may influence each other. Conventional regression analysis is not the appropriate statistical tool to evaluate the effect of different variables in such systems. In a multiple regression the structure of the model is fixed, allowing only for direct effects between independent and dependent variables. Correlations between the independent variables are left unanalyzed. However, this technique has often been used on data from regional surveys, with the result that potentially structurally complex relationships are presented in a simplified form. We believe that path analysis (Wright 1934) is better suited to study such a complex problem. In a path analysis, one makes explicit assumptions about the nature and directions of the causal relationships, and the causal effects of the explanatory variables are broken down into indirect and direct components. We use this approach in the present study and model how deposited mercury may enter lakes and accumulate in fish.

Materials and Methods

Data from Lakes

This study was based on data from 25 lakes that comprised a subsample from a national survey of 210 lakes investigated for heavy metal contamination in sediments (Rognerud and

TABLE 1. Variables describing the lakes. n = 25, SD = standard deviation of the mean.

Variable	Abbreviations	Units of measure	Mean	SD	Median	Range
Mercury in mosses	Hg_m	ng·g DW^{-1}	97	28	90	70–160
Mercury in sediments	Hg_s	ng·g DW^{-1}	185	118	160	40–440
Selenium in mosses	Se_m	µg·g DW^{-1}	0.65	0.39	0.50	0.16–1.45
Total organic carbon in water	TOC	mg·L^{-1}	2.4	1.5	2.0	1.0–6.0
Loss of ignition in sediments	LOI	%	30.0	9.6	29.0	15.0–52.0
pH		pH	6.20	0.42	6.30	4.94–6.65
Altitude	alt.	m	238	310	100	8–1125
Catchment area	A_c	km^2	33.6	48.0	16.3	1.5–192
Lake area	A_l	km^2	2.41	3.90	1.30	0.02–19.87
Maximum depth	z_m	m	30	17	30	8–64
Areal water load	q_s	m·yr^{-1}	22.5	24.9	14.2	1.6–97.6
Mean air temperature in summer	temp.	°C	12.8	2.2	13.5	8.2–15.9

Fjeld 1990). All the lakes were typically dimictic, oligotrophic, and with low alkalinity. This is the predominant type of lake in Norway. None of the lakes has any known point source of mercury. In Norway, there is approximately a south–north gradient in the long-range atmospheric deposition of pollutants, with the coastal areas in southern Norway receiving the greatest load of heavy metals and acidifying compounds (Rühling et al. 1987; Henriksen et al. 1988). However, this south–north gradient is less pronounced for mercury, as in the most northern part of Norway, there is a tendency toward higher values in coastal areas compared with further inland (Steinnes and Andersson 1991). Twenty of the lakes (lakes 6–25) were situated in southern Norway in boreonemoral, north-boreal, and low-alpine regions (Fig. 1). The five others (lakes 1–5) were located in northern Norway, in the county of Finnmark, a north-boreal to low-alpine region. By choosing lakes from different geographical regions, a broad range of mercury loadings was assured.

We included data on 12 variables in the statistical analysis (Table 1). The variables were chosen to represent overall physical and chemical characteristics of the lakes; many of them are either known or suspected to affect the bioaccumulation of mercury. The number of variables was kept low to reduce the chance for spurious correlations and to avoid overfitting of data. As we have no estimates of lake volumes, we could not calculate the lake water residence time. As a substitute for this, we have used the areal water load (q_s). This is defined as the annual water discharge from the catchment area divided by the lake area. Because of the correlation between lake area and volume, q_s expresses some of the variation in the lake water residence time.

The sediment and water samples were collected in the falls of 1986–88. A mixed water sample from epilimnion was collected from each lake, and at the same time, a sediment core was taken from the deepest point. The sediment corer was an open barrel gravity type, made of Plexiglas, with an automatic closing diaphragm. The sediment cores were sectioned after sampling, put in acid-washed polyethylene cups, deep-frozen, and sent to the laboratory for analysis. In this study, we have used the data from the upper 1-cm layer. The sedimentation rates in nordic forest lakes have been surveyed by several authors (El-Daoushy and Johansson 1983; Tolonen and Jaakkola 1983; Rekolainen et al. 1986; Renberg 1986). The mean sedimentation rate of surface sediments varies between 0.7 and 1.7 mm·yr^{-1}. Thus, the majority of our surface sediment samples are likely to have been deposited within the last decade.

The mercury and selenium contents of terrestrial feather mosses (*Hylocomium splendens*) were used as relative measures of the depositions rates for these elements. Data were provided by E. Steinnes (pers. comm.) and are estimates based on observations from representative locations nearby. Each sample consisted of several 1-yr-old buds, collected in 1985. The sampling procedures and a discussion of the use of feather mosses as indicators of heavy metal deposition are given in Steinnes and Andersson (1991).

After acid digestion, mercury in sediments and mosses was analysed with a cold vapour atomic absorbtion spectrophotometer equipped with a hydride generator (Hatch and Ott 1968). The detection limits were 40 and 20 ng·g dry weight $(DW)^{-1}$, respectively, for sediments and mosses. Selenium in mosses was analysed by instrumental neutron activation (INAA), with a detection limit of 20 ng·g DW^{-1} (Steinnes 1967). Total organic carbon (TOC) was analysed with a TOC-analyzer (Astro 2850). Loss on ignition (LOI) in sediments was calculated after drying at 60°C and ignition at 550°C.

We have used mean summer temperatures in air (average for June, July, and August) as a relative measure of epilimnic summer temperatures. The data are based on the current 30-yr normal temperatures from nearby weather stations (Bruun 1967) and are adjusted for differences in altitude between the lakes and weather stations.

Fish Collection and Mercury Analysis

All the lakes contained naturally reproducing populations of nonanadromous brown trout. Approximately 20 trout were caught with gill nets from each lake during the fall of 1988. We concentrated our effort on catching fish 20–30 cm long. Trout of this size feed mainly on invertebrates. Total length, weight, sex, and stage of maturity were recorded and scales or otoliths were collected for age estimates. Approximately 40 g of bone and skinless dorsal axial muscle tissue was removed from each fish, put separately in polyethylene bags, and frozen for shipment to the Department for Pharmacology and Toxicology at the Norwegian College of Veterinary Medicine in Oslo. In the metal analysis laboratory, samples of approximately 1 g were digested in a 4:1 nitric – perchloric acid mixture before being analysed for total mercury. The analytical instrument was a cold vapour atomic absorption spectrophotometer equipped with a hydride generator. The detection limit was 0.01 mg·kg wet weight $(WW)^{-1}$. The analyses were run in series of 40 samples, of which one sample was a standard

TABLE 2. Correlations between mercury concentrations in fish populations (log Hg_f) and the variables describing the lakes (abbreviations as in Table 1).

	log Hg_f	log Hg_m	log Hg_s	Se_m	log TOC	LOI	pH	log alt.	log A_c	log A_l	log z_m	log q_s	temp.
log Hg_f	1.00												
log Hg_m	0.37	1.00											
log Hg_s	0.62	0.58	1.00										
Se_m	0.34	0.70	0.60	1.00									
log TOC	0.78	0.41	0.46	0.52	1.00								
LOI	0.58	0.36	0.47	0.34	0.50	1.00							
pH	−0.10	−0.56	−0.43	−0.29	0.06	−0.33	1.00						
log alt.	−0.22	−0.34	−0.53	−0.64	−0.24	−0.19	0.22	1.00					
log A_c	−0.29	−0.15	−0.10	−0.33	−0.47	−0.41	−0.15	0.31	1.00				
log A_l	−0.21	−0.14	−0.10	−0.40	−0.52	−0.38	−0.23	0.23	0.79	1.00			
log z_m	−0.14	0.00	0.36	0.00	−0.38	−0.17	−0.16	−0.10	0.29	0.37	1.00		
log q_s	−0.15	0.04	0.17	0.14	−0.13	−0.09	−0.11	−0.11	0.41	−0.14	0.05	1.00	
temp.	0.70	0.64	0.75	0.78	0.68	0.53	−0.31	−0.59	−0.25	−0.29	0.08	0.15	1.00

reference and three were blanks. The standard reference was a fish homogenate (MAA2, produced by IAEA, delivered by Analytical Standards, Kungsbacka, Sweden). The specified Hg concentration of this reference material (0.47 ± 0.02 $mg \cdot kg^{-1}$) is an average, based on analyses from several authorized laboratories. Series with standard reference values outside the control limits (± 2 SD) were reanalysed. The record of the laboratory shows more than 95% of the analyses of standard references to be inside these limits.

Statistical Analysis

We checked the distributions of the data before the analysis, and all the variables except pH, q_s, Se_m, and LOI were log-transformed to improve normality. In order to ease comprehension of the text, we have omitted the transformations when referring to the variables. Our strategy for data analysis was first to estimate standardized mean mercury concentration for each population, adjusted for differences in length or age. We studied the relationship of the estimated mean fish mercury concentrations with different environmental variables by examining scatter plots and the produce-moment correlation matrix. We then screened potential predictor variables for fish mercury concentrations by inspection of their partial correlations and three-dimensional scatter plots. We have presented the correlation matrix for the whole data set in Table 2 to enable others to make a post hoc analysis of our data.

Based on the findings from the exploratory data analysis, and guided by current biological and limnological knowledge, we constructed a causal model and tested our hypotheses with multiple regressions and path analysis. We inspected the residuals and leverage plots from the regression analyses to ensure normality, linearity, and homeoscedastisity. To measure the relative importance of the independent variables, we used their squared semipartial correlation coefficients. This expresses the unique contribution of an independent variable to the total variance of the dependent variable (Tabachnick and Fidell 1989). We used a standard regression procedure, avoiding the frequently used technique of stepwise regressions, which is unreliable for making statistical inferences (Wilkinson 1992).

For the path analysis, we formulated a structural model a priori by postulating causal links between the predictor and criterion variables. We then estimated path coefficients for the predictor variables and examined the solution by inspection of their standard errors, the coefficient of determination, and a goodness-of-fit index (GFI: Jöreskog and Sörbom 1989). The path coefficients are the standardized regression coefficients for the structural equations representing the model. They do not express the rate of change in terms of their original measurement units, but in standard deviations. They are therefore a useful relative measure of the effects that predictor variables have on a criterion variable. The effects of a predictor variable may be both direct and indirect. The indirect effect along a path equals the product of the path coefficients. The total effect is the sum of direct and indirect effects. For an introduction to path analysis, see Sokal and Rohlf (1981) or Kingsolver and Shemske (1991) and references therein.

For the exploratory data analysis and the multiple regressions, we used the computer program SYSTAT for Macintosh (Wilkinson 1992). Traditionally, path analysis for directly observed variables is performed by a series of standardized multiple regressions. However, the computer program LISREL 7 (Jöreskog and Sörbom 1989) uses the covariance matrix to estimate all the model parameters simultaneously, and the program provides several powerful tools for an assessment of fit of a model. We have chosen to present a χ^2 statistic and a GFI. The χ^2 statistic tests the restricted model against the alternative of an unconstrained model. An underparameterized model will have a larger χ^2-value than a model with an optimal fit. The probability level associated with the χ^2-value is the probability of obtaining a larger value, given that the model is correct. The program also estimates modification indices that predict the reduction in χ^2 if a single constraint is relaxed and the model is reestimated. The GFI gives another overall measure of fit; the index varies from 0 to 1, where well-fitted models have a value close to 1. A more technical discussion of these measures of fit is provided in Jöreskog and Sörbom (1989).

Results

Mercury Concentrations in Fish

Mean mercury concentrations in fish from the various populations varied greatly, ranging from 0.01 to 0.24 $\mu g \cdot g^{-1}$, with the highest values in lowland lakes in the south (Fig. 2). There was a positive linear relationship between mercury concentrations and fish length in most populations (both variables transformed to $\log_{10}$). A model with the lake as a nominal independent variable and length as a covariate explained 88% of the variation in fish mercury concentration (ANCOVA: $F = 64.82$, df = 49/433, $p < 0.0001$). Both main effects and interactions were significant, indicating

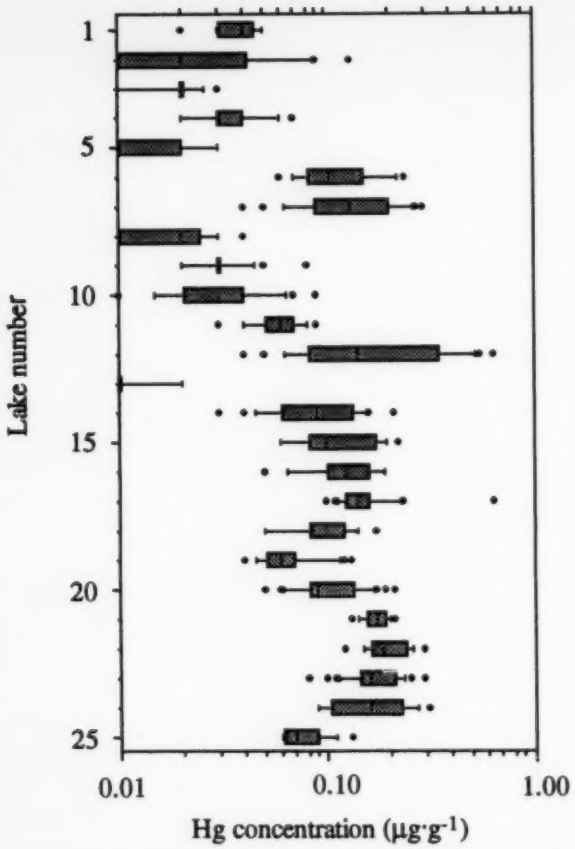

FIG. 2. Box-and-whisker plot showing the distribution of mercury concentrations in the populations of brown trout. Median values are represented by vertical lines, upper and lower quartiles are represented by boxes, and the 10th and 90th percentiles are represented by whiskers. For location of lakes, see Fig. 1.

heterogeneous regression slopes. Including age in the model led only to a minor increase in explained variation (≈1%). We decided therefore to adjust mean mercury concentrations only for differences in length. We used the adjusted means from the covariance analysis, which are adjusted to the geometric mean length for the whole sample (249 mm). Sex and stage of maturity did not influence mercury concentrations (ANCOVA).

Correlations

The length-adjusted means of fish mercury concentration were highly correlated with the TOC content of water, the mercury concentrations in surface sediments, and the LOI of sediments ($r > 0.58$). These were obvious candidates for predictor variables in a regression analysis, as we have reason to believe that causal mechanisms lie behind these correlations. The correlations of fish mercury concentration with mercury in terresterial mosses were modest ($r = 0.37$). However, several variables were intercorrelated with sediment mercury and TOC ($r > 0.40$). Such intercorrelations could mask the effects that these variables have on fish mercury concentration. To reveal such effects, we computed partial correlations for these variables. These demonstrated that selenium in mosses had a pronounced influence on fish mercury concentration when we controlled for the effect of TOC and sediment mercury ($pr = -0.42$). Neither pH, temperature, nor any other variables had similar effects. The analysis also showed that the correlation between fish mercury and ignition loss *could* be spurious and may be attributed to the intercorrelations of ignition loss with TOC and sediment mercury. The negative partial correlation for selenium indicates that high deposition rates of selenium were associated with low fish mercury concentrations, when TOC and sediment mercury were held constant. Inspection of three-dimensional scatter plots confirmed this (Fig. 3). Among the lakes with high values for both sediment mercury and TOC, we found that fish mercury concentrations were generally lower in areas with high selenium values. We chose these three variables as candidates for explanatory variables describing fish mercury concentration.

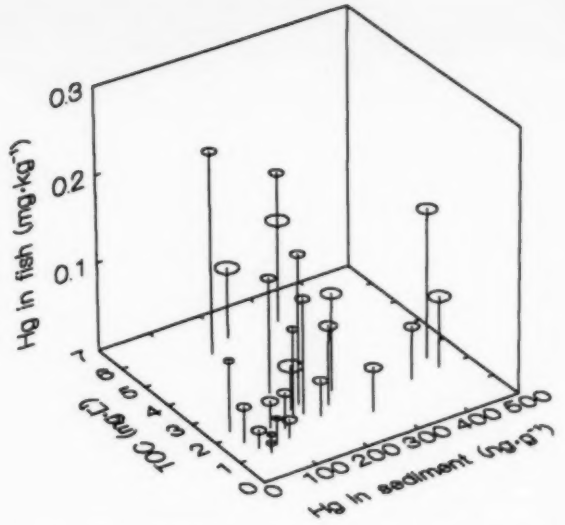

FIG. 3. Three-dimensional scatter plot of the relationships among adjusted means of fish mercury concentrations, TOC in lake water, and mercury in surficial lake sediments. The area of the circles is proportional to the concentration of selenium in mosses (0.16–1.45 $\mu g \cdot g^{-1}$).

The correlation matrix (Table 2) also indicates that sediment mercury concentrations could be explained by a set of variables with known causal effects, and we used TOC, mercury in mosses, and maximum lake depth as candidates for explanatory variables in a model describing the sediment mercury concentrations.

Causal Modelling and Path Analysis

Based on the results from the explorative data analysis, we developed a causal model with mercury in sediments (Hg_s), TOC, and selenium (Se_m) as explanatory variables with direct effects on the fish mercury concentrations (Hg_f). We incorporated a submodel describing the variance in sediment mercury by allowing for indirect effects of mercury in mosses (Hg_m), TOC, and lake depth (z_m) on fish mercury. This causal model is a recursive system with four exogenous explanatory variables (x_n), two jointly dependent variables (y_n), and two random error variables (ζ_n) (Fig. 4). The error variables are composite vari-

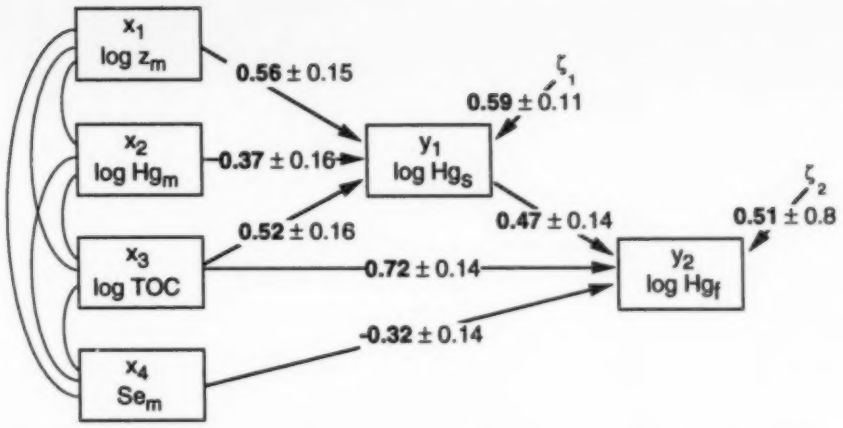

FIG. 4. Path diagram for the model describing mercury concentrations in brown trout populations. Arrows between variables indicate suggested causal effects; noncausal covariation is illustrated by curved lines. The path coefficients (with standard errors), which depict the relative magnitude of the effects, appear on the arrows. Residual effects are symbolized with the greek letter ζ. For an explanation of other variable names, see Table 1.

TABLE 3. Standard multiple regression between fish mercury concentration (log Hg_f) as dependent variable and environmental variables. $n = 25$, SE = standard error, sr^2 = squared semipartial correlation. $R^2 = 0.74$, $R^2_{adj} = 0.71$; ANOVA: $F = 20.38$, df = 3/21, $p < 0.0001$. See Table 1 for abbreviations of variables.

Term	Coefficient	SE	t	p	sr^2
log Hg_s	0.547	0.163	3.35	0.003	0.14
log TOC	1.129	0.207	5.45	<0.0001	0.36
Se_m	−0.293	0.134	−2.19	0.040	0.06
Intercept	−2.583	0.309	−8.37	<0.0001	

ables, made up of all residual causes associated with the dependent variables. The structural equations are

(1) $y_2 = \beta_{21}y_1 + \gamma_{23}x_3 + \gamma_{24}x_4 + \zeta_2$
(2) $y_1 = \gamma_{11}x_1 + \gamma_{12}x_2 + \gamma_{13}x_3 + \zeta_1$.

The regression coefficients for the three independent variables in Eq. 1 were all statistically significantly different from zero, and the regression model described 75% of the variance in the mercury concentration in fish (Table 3). The squared semipartial correlations showed that TOC had the greatest unique contribution with 36%, the contribution from Hg_s was 14%, and Se_m had the lowest with 6%. This leaves 18% of the explained variance as a shared contribution, probably mainly attributable to the intercorrelated variables TOC and Hg_s. By excluding selenium from the independent variable set, the explanatory power of the variable Hg_s dropped considerably; its unique contribution was reduced to 8%. By excluding Hg_s from the variable set, we reduced the unique contribution of Se_m to nearly zero. Thus, selenium acts as a suppressor variable (sensu Tabachnik and Fidell 1989), and its effect was mainly to suppress variance in Hg_s that was irrelevant for predicting fish mercury concentrations.

The regression analysis of Eq. 2 showed all regression coefficients to be significantly different from zero (Table 4). The model explained 65% of the variance of mercury in sediments. The semipartial correlations showed lake depth to be the most important variable, having a unique contribution of 28%. The variables TOC and Hg_m contributed 16 and 10%, respectively. Thus, 9% of the explained variation was due to a shared contribution.

Examination of the solution of the path analysis showed the parameter estimates (path coefficients, Fig. 4) and effect coefficients to have acceptable standard errors, and modification indices indicated that the model would not be substantially improved by allowing for other effects between the variables. The total coefficient of determination for the structural equations was high ($R^2 = 0.86$), and the overall fit of the model was good: $\chi^2 = 1.22$, df = 3, $p = 0.75$; GFI = 0.98. TOC was the variable with the greatest effect on fish mercury concentrations. It had a direct effect of 0.72 (γ_{23}) and an indirect effect of 0.26 ($\gamma_{13}\ \beta_{21}$), giving a total effect of 0.97 (SE ± 0.16). Thus, a change of 1 SD unit in TOC results in a change of 0.97 SD unit in Hg_f. The total effect of Hg_s was substantially lower, with an effect coefficient of 0.47 (±0.14). The total effects of lake depth and selenium were about of the same magnitude, but with opposite signs: 0.26 (±0.15) and −0.32 (±0.14), respectively. The Hg_m had the lowest total effect, with a coefficient of 0.17 (±0.09).

Discussion

In this study, we have demonstrated that the mercury levels in Norwegian populations of brown trout vary regionally in a pattern roughly corresponding to the atmospheric deposition of mercury. In the pollution-exposed areas of southern Norway,

TABLE 4. Standard multiple regression between sediment mercury concentration (log Hg_s) as dependent variable and environmental variables. $n = 25$, SE = standard error, sr^2 = squared semipartial correlation. $R^2 = 0.65$, $R^2_{adj} = 0.60$; ANOVA: $F = 13.19$, df = 3/21, $p < 0.0001$. See Table 1 for abbreviations of variables.

Term	Coefficient	SE	t	p	sr^2
log Hg_m	0.959	0.374	2.561	0.018	0.14
log TOC	0.705	0.209	3.370	0.003	0.29
log z_m	0.611	0.155	3.936	0.001	0.06
Intercept	−0.807	0.712	−1.134	0.270	

the length-adjusted population means were generally 3–5 times higher (mercury concentrations in muscle: 0.10–0.15 μg·g WW^{-1}) than in the more unexposed northern areas. However, correlations revealed that less than 14% of the observed variation could be attributed to atmospheric deposition rates alone (indirectly measured as mercury concentration in terrestrial mosses). Our results from the causal modelling and path analysis support the view that other local environmental factors also are involved. In our model, we have allowed for effects from variables that we believe are involved in the transport of mercury to the lakes, sedimentation processes, and the production of methyl mercury.

The bioavailability of mercury depends greatly on its speciation. When methylated, mercury accumulates readily in biota, with as much as 99% of the mercury in fish existing in this form (Grieb et al. 1990). Fish do not methylate mercury in vivo but accumulate MeHg from both food and water (Pennacchioni et al. 1976; Huckabee et al. 1978). The production of MeHg has traditionally been attributed to microbially mediated processes occurring in lake sediments and water (Furutani and Rudd 1980; Korthals and Winfrey 1987; Xun et al. 1987; Parks et al. 1989). Abiotic methylation where fulvic acids act as methyl donors is also known (Rogers 1977; Nagase et al. 1982; Lee et al. 1985), but as compared with biochemical methylation, the environmental significance of such abiotic intralake methylation is minor (Berman and Bartha 1986). However, recent advances in analytical methods have led to the detection of significant amounts of MeHg in runoff from catchment areas and in rain (Lee and Hultberg 1990; Lee and Iverfeldt 1991). Although the reported ratio of MeHg to total mercury is low (1–6% in runoff, 0.1–4% in rain), the estimated annual input of MeHg may be large enough to contribute substantially to the amount accumulated in fish populations. As there exist no estimates of the amount of MeHg produced within such drainage lakes, it is difficult to assess the actual importance of allochthonous versus autochthonous MeHg. However, studies of seepage lakes, newly flooded reservoirs, and drainage lakes with sediments contaminated by historical pollutions leave no doubt as to the significance of the methylation processes in lakes (Lodenius et al. 1983; Rudd et al. 1983; Jackson 1988; Parks et al. 1989; Bloom and Effler 1990; Wiener et al. 1990; Fitzgerald et al. 1991; Verdon et al. 1991).

The methylation rate in lake water and sediments increases with mercury concentration (Jensen and Jernlöv 1969; Rudd et al. 1983; Xun et al. 1987). The maximum rate occurs at the sediment–water interface, decreasing in the overlying water column and in subsurface sediments (Rudd et al. 1983; Korthals and Winfrey 1987). The release of MeHg from the surface sediments into the water column may be favoured by short diffusion distances and mechanical disturbances in sediments such as redistribution and bioturbation. As in our survey, others have also found significant correlations between the mercury concentrations in surface sediments and fish (Andersson et al. 1987; Johnson 1987; Cope et al. 1990). We therefore believe that it is justified to suggest that the effect from Hg_s to Hg_f in our model reflects a concentration-dependent production of MeHg in the surface sediments.

In our model, we let three factors influence the mercury concentration in the surface sediments: mercury concentrations in terrestrial mosses (*H. splendens*), the depth of the lake, and TOC in the lake water. We have used the mercury concentration in mosses as a relative measure of the atmospheric deposition of this element. According to Steinnes and Andersson (1991), mercury concentration in *H. splendens* is an appropriate indicator. Atmospheric input is the major source of mercury in terrestrial and aquatic ecosystems, and mass balance studies of Swedish drainage lakes show that substantial amounts are supplied to lakes by both direct deposition on the lake surface and runoff from catchment areas (Johansson et al. 1991; Meili 1991).

We believe that the effect of TOC on the sediment concentrations in our model most likely reflects the influence of humic matter on the transportation of mercury into the lake whereas the effect of depth probably expresses depth-dependent retention and sedimentation of mercury. Humic matter is an effective metal complexing agent of di- and trivalent metal ions (Schnitzer and Khan 1972), and the atmospheric depositions of mercury, which are mainly inorganic Hg(II) ions or complexes (Iverfeldt 1991), are partly retained in catchment areas (Johansson et al. 1991). The humic layer here acts as a repository, supplying mercury to the aquatic environment. Transport to the lakes peaks during flooding, when the concentration of humic matter in the runoff is high (Borg and Johannson 1989; Lee and Hultberg 1990). Mercury is linked to carbon cycling in lakes because of its association with humic matter, and the element follows fine organic particles and humic colloids in their aggregation and sedimentation processes (Santschi 1988). The retention of mercury will therefore be greater in deep lakes than in more shallow ones with short water residence time. Moreover, resuspension and resedimentation of sediment will cause a continual size-selective sorting and transportation of fine-grained particles from shallow areas to the deeper parts of the lake (the funneling effect: Ohle 1962; Håkanson and Jansson 1983). In our model, we have incorporated the influence of these two processes by permitting a direct effect of lake depth on sediment concentrations of mercury.

In addition to the indirect effect of TOC on fish mercury in our model, we have also included a direct effect, as our data strongly support. We believe that this effect may be due to two important processes. First, it may simply reflect association between humic matter and mercury, both allochthonous MeHg

and inorganic species (Lee and Hultberg 1990; Lee and Iverfeldt 1991). Second, TOC may indirectly change the speciation of mercury by serving as a growth substrate for mercury-methylating bacteria, thus speeding up the net methylation rate. Experiments have shown that the addition of biodegradable organic matter to lake water and sediments may have such effects (Furutani and Rudd 1980; Rudd et al. 1983; Jackson 1988, 1989; Hecky et al. 1991). It has also been suggested that the often-observed correlations between dissolved organic carbon (DOC) and fish mercury may partly be due to this (Verta 1984; Mannio et al. 1986; McMurty et al. 1989; Wren et al. 1991). However, recent studies show that the role of DOC may be more ambiguous. Miskimmin et al. (1992) demonstrated in vitro that naturally extracted DOC from an outlet of a sphagnum bog had an inhibitory effect on the net methylation rate under aerobic conditions. The authors believe that the most likely explanation is that the availability of Hg^{2+} is reduced by the binding of free mercury ions to humic matter. This theory is supported by the finding of a negative correlation between fish mercury and DOC in seepage lakes (Cope et al. 1990; Grieb et al. 1990). These apparently inconsistent effects of DOC may partly be due to its heterogenous nature. DOC in natural waters is composed of two distinct pools: nonhumic labile and humic refractory. The relative size of these two pools, as well as their bioavailability, may vary substantially between different lake types (Moran and Hodson 1990). The composition of DOC should therefore determine the influence it will have on microbial methylation activity and the fraction of mercury ions available for methylation. But, if DOC generally hinders the in-lake methylation processes, as Miskimmin et al. (1992) suggested then the correlation between DOC and mercury in fish may indicate that input of allochthonous MeHg is critical for the mercury accumulation in lake ecosystems. However, to better clarify the biological significance of in-lake versus extra-lake methylation, more knowledge about the function of DOC in the methylation processes is required.

We were somewhat surprised to find that the depositions of selenium could be used to explain the variation in fish mercury concentrations. Additions of selenium to aquatic ecosystems have been shown to interfere with the accumulation of mercury in freshwater fish (Rudd et al. 1983; Turner and Swick 1983; Paulsson and Lundbergh 1991), but to our knowledge the present study is the first indicating that long-range-transported selenium may have such effects. How selenium may influence fish mercury concentration is not well understood. Selenium is a biologically active element known to interact with the metabolism of heavy metals (Whanger 1981). It accumulates along ecological food chains, and Turner and Swick (1983) found that elevated selenium concentrations in food resulted in a less efficient mercury uptake in fish. It has also been suggested that selenium may lower the fraction of mercury available for methylation by reacting with free Hg^{2+} and forming the nearly insoluble HgSe ($K_s = 10^{-58}$) (Björnberg et al. 1988). However, we have no basis for evaluating the mechanisms possibly involved in our study.

We could not detect any significant effect of pH on mercury concentrations in trout. In fact, we could not find any subset of explanatory variables where pH contributed significantly. This is contrary to a number of other studies, where pH is regarded as one of the key factors influencing mercury accumulation in freshwater fishes. The proposed mechanism is a pH-dependent stimulation of net methylation rates (Winfrey and Rudd 1990). The reason why pH had no detectable effect in our model may be because the lakes we studied were distributed within a relatively narrow pH range. The pH in most of our lakes varied between 5.7 and 6.7, which is the typical pH range for trout lakes in Norway. It may be difficult by statistical methods to demonstrate an effect of pH when it can only be studied within such a narrow range; the effect may simply be too small to discriminate from other random effects. We therefore believe that there is not necessarily any conflict between our results and those of studies that conclude that pH influences the bioaccumulation of mercury.

Our rather simple model demonstrates several advantages of causal modelling and path analysis, as compared with multiple regressions. For our data set, a conventional multiple regression approach would have left us with the reduced model expressed by Eq. 1. No distal factors would have been included, and the effect of TOC on sediment mercury would have been left as an unanalysed correlation. Thus, the effects of atmospheric deposition rates and lake depth would have been retained and hidden in the mercury sediment concentrations, and the effects of TOC would have been underestimated.

When evaluating the results of a path analysis, as well as other correlative techniques, one must bear in mind that the findings of a statistically significant effect do not in themselves establish a causal relationship. Such can only be demonstrated by experimental proof. Path analysis is primarily a method for tracing out the implications of the set of causal assumptions one is willing to impose on a system of relationships. However, carefully combined with a judgmental evaluation of the results from other studies, path analysis can be used to make causal inferences. Where causal relationships are established, the technique may be used to evaluate the relative importance of different causal pathways. Path analysis has now been elaborated to include the study of latent, not directly observed variables (Jöreskog and Sörbom 1989). When reliable environmental indicators of microbial methylation rates are established, we suggest that this technique could be used for assessing the importance of allochthonous versus autochthonous MeHg. Also, we believe that there is a rich source of information in existing databases that could be further utilized in path analysis studies for gaining insight into the distribution of environmental pollutants.

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Homing and Straying Patterns of Chinook Salmon (*Oncorhynchus tshawytscha*) from a New Zealand Hatchery: Spatial Distribution of Strays and Effects of Release Date

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Homing and straying patterns of fall chinook salmon (*Oncorhynchus tshawytscha*) released from the Glenariffe Salmon Research Station on the Rakaia River, New Zealand, are reported, based on coded-wire tag recoveries from the 1978–84 brood years. Of 17 671 tagged adults recovered, 87.9% returned to the Rakaia, and the rest were recovered from 12 other catchments up to 500 km away. The number of strays entering a given river increased with discharge and with proximity to the Rakaia, but most strays were recorded in catchments north of the Rakaia. A higher proportion of salmon released in winter, when the downriver migration of naturally produced chinook is a minimum, strayed to other catchments (14.9–20.6%) than did those released at other times of the year (3.6–7.6%). However, straying within the Rakaia catchment was largely unaffected by release date, suggesting that imprinting by fry to the natal tributary is separate from imprinting by smolts to the mainstem river. There was a complex interaction between age at maturity, release date, and straying rates; straying of 4-yr-old fish was more strongly influenced by release date than straying of younger fish. Notwithstanding this interaction, straying was more prevalent among older fish.

Nous présentons les schémas de retour et de dispersion des saumons quinnats (*Oncorhynchus tshawytscha*) d'automne libérés par la station de recherche salmonicole de Glenariffe, sur la rivière Rakaia, en Nouvelle-Zélande, à partir des récupérations de micromarques codées des années de reproduction 1978–1984. Sur 17 671 adultes marqués récupérés, 87,9 % sont revenus à la Rakaia, et les autres ont été récupérés dans 12 autres bassins versants pouvant être distants de 500 km. Le nombre d'individus égarés entrant dans un cours d'eau donné augmentait avec le débit et la proximité de la Rakaia, mais la plupart des saumons errants ont été signalés dans les bassins situés au nord de la Rakaia. La proportion de poissons égarés vers d'autres bassins était plus forte parmi les saumons libérés en hiver, lorsque l'avalaison des quinnats produits naturellement est au minimum (14,9–20,6 %) que parmi les saumons libérés à d'autres périodes de l'année (3,6–7,6 %). Toutefois, à l'intérieur du bassin de la Rakaia, la dispersion était peu affectée par la date de libération, ce qui peut indiquer que l'empreinte de l'affluent natal chez les alevins est différente de l'empreinte du cours d'eau principal chez les smolts. On a observé une interaction complexe entre l'âge à la maturité, la date de libération et le taux de dispersion; le nombre d'individus égarés chez les poissons de 4 ans était plus fortement affecté par la date de libération que chez les poissons plus jeunes. En dépit de cette interaction, la dispersion était plus courante parmi les poissons âgés.

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Homing is one of the more important and well-studied characteristics of Pacific salmon (*Oncorhynchus* spp.). Homing has been demonstrated many times, both for native populations returning to a specific lake, stream, or hatchery (Quinn 1990) and for transplanted hatchery populations returning to their release point (Donaldson and Allen 1958; Vreeland et al. 1975). Although the mechanisms guiding the oceanic phase of homing remain unclear, the final freshwater stages of the migration are based on olfactory cues acquired by juveniles during their freshwater residence and downstream migration (Hasler and Scholz 1983).

In New Zealand, self-propagating chinook salmon (*O. tshawytscha*) stocks were established at the Hakataramea Hatchery on the Waitaki River during the early 1900s (McDowall 1990), following a series of importations of eyed ova from a tributary of the Sacramento River between 1901 and 1907. Within 15 yr of these liberations, salmon were caught "freely" in the Rangitata, Rakaia, and Waimakariri rivers (McDowall 1990), the mouth of the latter being over 230 km northeast of the Waitaki River mouth (Fig. 1). No other east coast rivers were stocked during this period, so the appearance of anadromous populations outside the Waitaki catchment can be attributed to straying over a period representing at most four or five generations.

The advent of coded-wire tagging (Jefferts et al. 1963), together with major hatchery releases of chinook and coho salmon (*O. kisutch*), and extensive tag recovery programmes

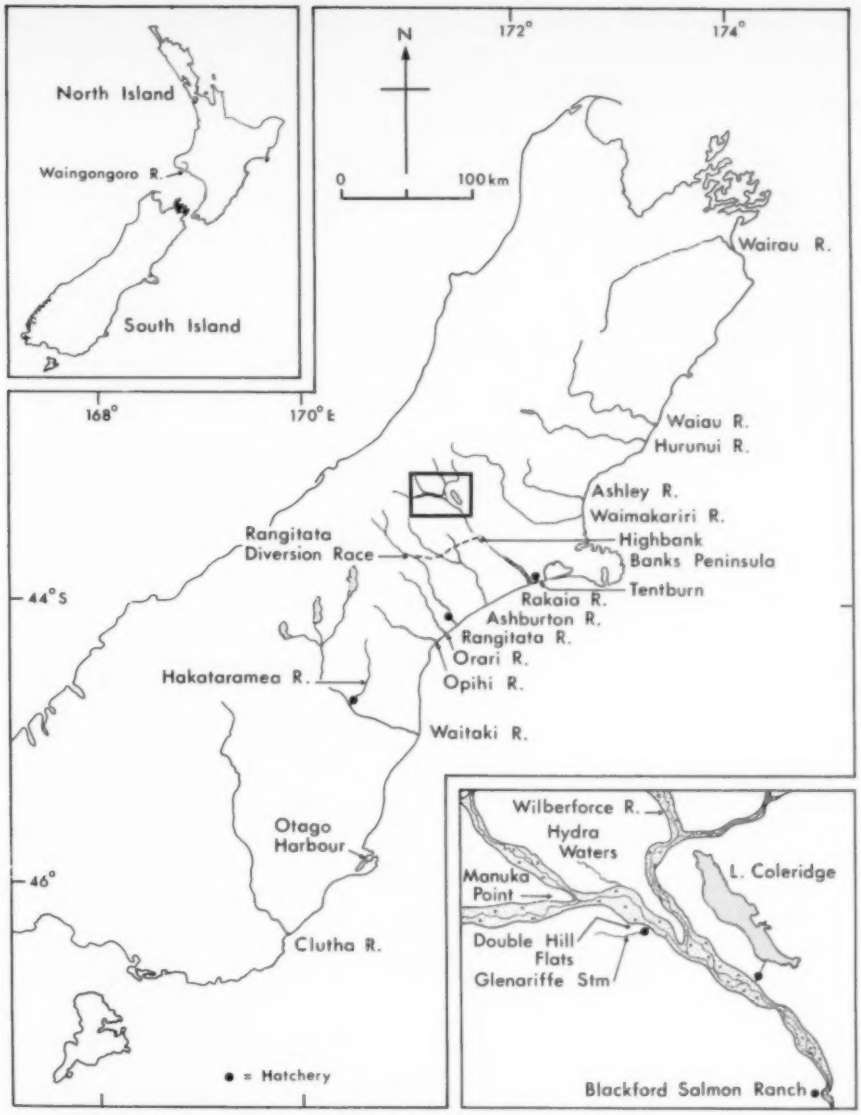

FIG. 1. South Island of New Zealand, showing localities referred to in the text. Major spawning streams within the Rakaia catchment (inset, lower right) are the Hydra Waters, Manuka Point, Double Hill Flats, and Glenariffe Stream. The hatchery on the Hakataramea River no longer operates.

(Johnson 1990), provides a rich source of data on homing and straying in North America. Coded-wire tag data, used to derive estimates of straying for chinook salmon released from hatcheries in the lower Columbia River system (Quinn and Fresh 1984; Quinn et al. 1991), revealed considerable variation in the proportion of strays in a given population, and even greater variation in the number of strays entering different rivers. The factors underlying the distribution of strays are not understood, but proximity to the natal site and some combination of flow, temperature, and habitat suitability may be involved.

While straying may be influenced by river characteristics such as flow and temperature, homing itself depends on olfactory information acquired by juvenile salmon during freshwater residence and seaward migration. There is controversy as to whether salmon imprint on home stream odours during only the smolt stage (Hasler and Scholz 1983) or in some sequence during development and/or downstream migration (Quinn 1990). However, it is generally accepted that the period of transition from freshwater to the ocean, known as smolting, is critical for imprinting and that imprinting is linked to elevated thyroid hormone levels (Hasler and Scholz 1983). To the extent that this is true, salmon entering the ocean during the season(s) normally associated with smolting and migration should show a stronger homing response than those released at other times of the year.

Since 1977, over 4.5 million coded-wire tagged chinook salmon have been released from hatcheries in New Zealand

(Unwin et al. 1988b). As part of experiments to determine the influence of size and season of release on survival, groups of coded-wire tagged salmon were released throughout the year. These releases provided the opportunity to examine the relationship between homing and season of release and to describe the general patterns of straying both between and within catchments in New Zealand.

Methods

Glenariffe Hatchery is located on Glenariffe Stream (Fig. 1), one of four major salmon spawning streams in the upper Rakaia River catchment (West and Goode 1987). The stream is predominantly spring fed (mean discharge 5 $m^3 \cdot s^{-1}$), with stable water temperatures averaging 7–8°C in winter and 10–14°C in summer (Unwin 1986). Prior to the commencement of hatchery operations in 1977, Glenariffe Stream supported an average spawning population of 1886 chinook (Flain 1982), considered (by virtue of their up-river migration in late summer and autumn) to be equivalent to North American fall-run stocks. Ova for the hatchery were taken exclusively from this population.

Salmon releases from Glenariffe for the 1978–84 brood years totalled 2.7 million fish, including 1 522 614 coded-wire-tagged fish in 154 groups. Prior to 1982, the release programme generally involved fish 3–6 mo old weighing 10–20 g (Unwin et al. 1988b). For the 1982–84 brood years, all releases were part of an experimental time and size at release study (Unwin et al. 1989) involving older (7–15 mo) and larger (up to 150 g) fish. All fish were tagged according to the procedures described by Unwin et al. (1987) and identified externally by removal of the adipose fin. Survival to maturity for these groups averaged 1.25% and ranged from 0.04 to 7.56%.

Between 1980 and 1989, 17 671 tagged adults, aged 2–5 yr, were recovered from hatcheries (60.4%), anglers (30.4%), spawning ground surveys (8.7%), and counting fences (0.5%) (Unwin et al. 1987). All tags were recovered from salmon returning to the hatcheries, but we did not expand the data to allow for unrecovered tags from anglers and spawning ground surveys because of uncertainties about the total number of salmon returning. Return data from anglers and spawning stream surveys thus provided conservative estimates of straying but were not biased with respect to release group.

For each tag group, we determined the rate of straying between catchments by partitioning all returns into two subgroups representing salmon homing back to the Rakaia River and those recovered in other catchments. Returns to the Rakaia River (15 527 fish) were subdivided into three further groups: (1) fish that homed, comprising those recovered either at the Glenariffe counting fence or from the 400 m of stream between the fence and the Rakaia confluence (10 300 fish); (2) fish that had demonstrated some tendency to stray within the Rakaia catchment, comprising returns from hatcheries other than Glenariffe (767 fish), carcases from spawning grounds other than Glenariffe Stream (1182 fish), and fish caught by anglers upstream of the Rakaia–Glenariffe confluence (6 fish); and (3) fish caught by anglers downstream of Glenariffe Stream, for which the subsequent homing could not be determined (3272 fish). Straying rates within the Rakaia catchment were calculated from the totals for groups (1) and (2). Because of the large uncertainties (coefficient of variation >50%) for straying rates estimated from tag groups with low return rates, we excluded 34 groups with fewer than 30 returns (comprising 584 fish) from analysis of straying rates for individual groups.

TABLE 1. Geographical distribution of recovered chinook salmon originating from Glenariffe Hatchery. Catchments are listed from north to south. Discharge figures are either for the river mouth or for the lowest available gauging station. Distances from the Rakaia River represent the shortest distance by sea to each river mouth.

River	Mean discharge ($m^3 \cdot s^{-1}$)	Distance and direction from Rakaia River (km)	Tags recovered	
			Number	% of total
Waingongoro	2.7	580 N	1	0.006
Wairau	114	370 N	3	0.02
Waiau	99.4	200 N	199	1.13
Hurunui	40.4	180 N	583	3.30
Ashley	14.6	150 N	2	0.01
Waimakariri	116	140 N	665	3.76
Tentburn	2–3	10 N	27	0.15
Rakaia	196	0	15 527	87.87
Ashburton	25	37 S	22	0.12
Rangitata	92	65 S	626	3.54
Orari	10.7	75 S	6	0.03
Opihi	30	80 S	3	0.02
Waitaki	350	145 S	7	0.04

In adopting the above definitions of straying, we acknowledge that there is room for debate over whether some recoveries (e.g., those intercepted at another hatchery) should be regarded as "strays" (cf. Quinn et al. 1991). In practice, there is likely to be a spectrum of homing behaviour, ranging from fish that return to their natal stream with no significant deviation through fish that locate their home stream only after several false leads to those that fail even to locate their home catchment. Since our main objective was to compare straying rates between release groups, we did not wish to reduce the size of our data set unnecessarily by adopting too restrictive a definition of straying. All of the fish we identified as strays exhibited some tendency to deviate, even if they might subsequently have homed had they been given the opportunity to do so.

We investigated the spatial distribution of strays and then examined the relationship between release date and straying for each age group by combining data for all tag groups released during each calendar month and tabulating these data against age at return and location of recapture. All 1982–84 brood releases were conducted at 30-d intervals, on the same dates each year.

Results

Geographical Distribution of Strays

Of the 17 671 tagged salmon recovered, 15 527 (87.9%) returned to the Rakaia catchment (Table 1). The remaining 2144 (12.1%) were recovered from 12 catchments over 720 km apart, from the Waingongoro River in the North Island to the Waitaki River, 145 km south of the Rakaia River. However, virtually all strays (99.8%) were recovered between the Waiau and Rangitata rivers (Fig. 1).

The number of strays recorded in any one catchment varied greatly and seemed to be related both to river discharge and geographical location. Most strays entered theWaiau, Hurunui, Waimakariri, and Rangitata rivers, all relatively large rivers with established salmon runs, draining into the sea within 200 km of the Rakaia mouth. Smaller rivers such as the Ashley,

TABLE 2. Geographical distribution of Glenariffe Stream chinook salmon within the Rakaia River catchment, based on 12 255 tag recoveries from hatcheries (86.7%), spawning ground surveys (11.2%), anglers fishing at or upstream of the Glenariffe confluence (1.7%), and miscellaneous sources (0.4%).

Location	Distance from river mouth (km)	Tags recovered	
		Number	%
Mainstem below gorge	20–60	8	0.07
Highbank tailrace	52	34	0.28
Blackford Salmon Ranch	66	37	0.30
Mainstem above gorge	70–95	6	0.05
Coleridge Hatchery	82	730	5.96
Wilberforce River	90	3	0.02
Glenariffe Hatchery	95	9856	80.42
Glenariffe Stream	95	444	3.62
Double Hill Flats Stream	96	1047	8.54
Hydra Waters	100	81	0.66
Manuka Point	110	9	0.07

Ashburton, Orari, and Opihi attracted few strays, as did the two most distant rivers (the Wairau and Waingongoro).

We modelled the number of strays N entering each catchment as a function of mean annual discharge Q and distance from the Rakaia D, using relationships of the general form

$$(1)\quad N = \alpha Q^{\beta} e^{-\gamma D^{\lambda}}$$

or equivalently

$$(2)\quad \ln(N) = \ln(\alpha) + \beta \ln(Q) - \gamma D^{\gamma}$$

from which the coefficents α, β, and γ can be estimated by linear regression. For positive α, β, and γ, equation (1) predicts that the number of strays entering a given river should decrease with increasing distance from the Rakaia, but increase with discharge. We used the parameter λ to explore the effect of replacing D by power transformations such as $D^{1/2}$, D^2, etc., thereby altering the rate at which straying decreases with distance. Although this model seems intuitively reasonable, the best fit to the data we could produce ($\lambda = 0.25$; $\alpha = 119.6$, $\beta = 0.803$, $\gamma = 1.313$; $F_{2,10} = 5.87$, $p = 0.021$) explained little more than half the variance in $\log(N)$ ($r^2 = 0.540$). The main discrepancies from the model predictions were the Waimakariri and Hurunui rivers for which strays were underestimated by factors of 11 and 31, respectively, and the Opihi and Waitaki rivers (the two most southern rivers) for which strays were overestimated by factors of 12 and 20, respectively. Transforming the raw data by increasing strays to the south by a factor of 20 relative to those to the north improved the fit significantly ($\lambda = 0.5$; $\alpha = 92.4$, $\beta = 0.937$, $\gamma = 0.281$; $F_{2,10} = 10.05$, $p = 0.004$, $r^2 = 0.668$). Restricting the model to northern rivers also increased the correlation coefficient ($r^2 = 0.724$) at the expense of reducing the residual degrees of freedom from 10 to 5 ($\lambda = 0.5$; $\alpha = 39.7$, $\beta = 1.023$, $\gamma = 0.254$; $F_{2,5} = 6.56$, $p = 0.04$).

Within the Rakaia catchment, 84.0% of the salmon were classified as having homed; 80.4% entered Glenariffe Hatchery and 3.6% were recovered directly below the trap, either as spawned-out carcases or by anglers fishing at the confluence with the Rakaia (Table 2). Most of the remaining fish (14.5% of the total) were recovered at one of two locations: Double Hill Flats, a spawning ground 1 km upriver from Glenariffe (West and Goode 1987), or Coleridge Hatchery, which utilises water diverted from Lake Coleridge via a small hydroelectric plant

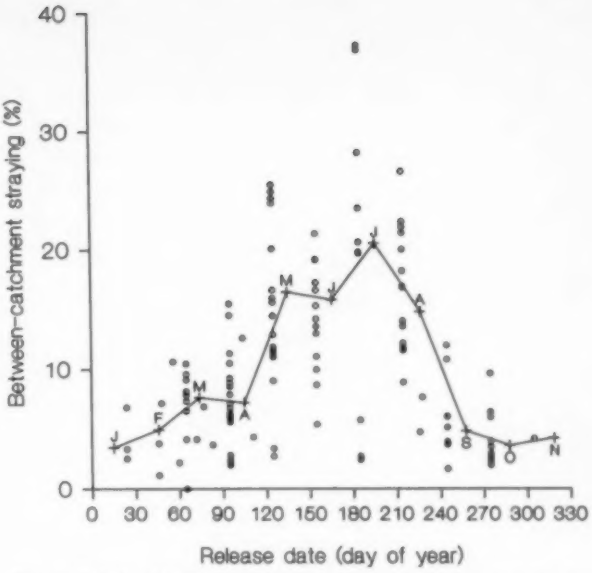

FIG. 2. Relationship between release date and straying rates to catchments other than the Rakaia for 120 groups of coded-wire-tagged chinook salmon (N = 17 119) released from Glenariffe Hatchery, 1978–84 brood years. The number of tags recovered per group ranged from 30 to 951, with a median of 89. Mean straying rates (+) for each month of release (indicated by first letter, excluding December) are indicated by the line.

13 km below Glenariffe. Other strays were recovered from the Hydra Waters, another spawning ground 5 km above Glenariffe, Blackford Salmon Ranch, a small hatchery 29 km below Glenariffe, and another small hydroelectric plant at Highbank, 43 km below Glenariffe (Fig. 1).

Influence of Release Date

Juvenile release date had a profound influence on straying. Straying between catchments was most common for salmon released during winter (June–August; Fig. 2), up to 37.4% from a release on 4 July 1984. Variation among months was highly significant ($\chi^2 = 538.1$, df = 11, $p < 0.001$). On average, 17.0% of the returning salmon released between May and August strayed to other catchments, compared with an average of 4.2% for releases between September and February.

Straying within the Rakaia catchment also varied significantly with release month ($\chi^2 = 114.4$, df = 11, $p < 0.001$), although the seasonal trend was less pronounced than for between-catchment straying (Table 3). Maximum straying for an individual coded-wire tag group (35.1%) was recorded for a release on 3 August 1983. Straying for spring and summer releases (September–February) averaged 11.4%, compared with 16.9% for autumn and winter releases (March–August).

Straying levels between and within catchments were poorly correlated ($r^2 = 0.237$), although straying to other catchments did not generally exceed straying within the Rakaia catchment (Fig. 3). The principal exceptions to this trend were winter releases in June and July for which between-catchment straying exceeded within-catchment straying by up to 20%. Defining "strong" homing as anything over 90%, we found that while strong homing to Glenariffe Stream almost always implied

TABLE 3. Straying rates for Glenariffe Stream chinook salmon within the Rakaia catchment as a function of age at return and month of release. Data shown for each month are the total number of tags recovered and the percentage of strays for all tag groups combined. Tags recovered from 5-yr-old fish are not tabulated but are included in the totals for each month.

Month of release	2-yr-olds		3-yr-olds		4-yr-olds		Total	
	N	%	*N*	%	*N*	%	*N*	%
January	30	10.0	220	12.3	23	4.3	273	11.4
February	28	10.7	275	14.9	21	0.0	324	13.6
March	166	18.1	1040	22.7	237	4.2	1444	19.1
April	267	11.2	1658	10.8	338	14.8	2264	11.4
May	126	8.7	1053	16.9	296	25.0	1475	17.8
June	63	12.7	628	11.1	311	25.1	1002	15.6
July	330	22.7	1058	15.1	354	30.5	1742	19.7
August	569	11.1	1151	20.6	513	23.0	2234	18.7
September	229	3.9	324	19.1	140	2.1	693	10.7
October	231	7.8	316	19.3	210	3.8	760	11.4
November	0	—	20	15.0	16	0.0	36	8.3
December	0	—	7	0.0	0	—	7	0.0

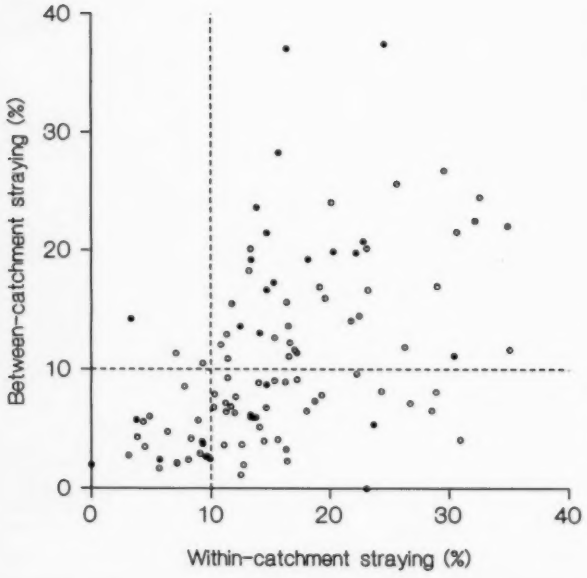

FIG. 3. Straying between catchments versus straying within the Rakaia catchment for the same data set as in Fig. 2. June and July releases are identified by solid circles. "Strong" homing between or within catchments is defined as those releases for which straying was <10% (broken lines).

equally strong homing to the Rakaia River, strong homing to the Rakaia catchment did not necessarily imply strong homing to Glenariffe Stream. Qualitatively, this is illustrated by the roughly triangular distribution of Fig. 3, with a distinct concentration of points (38 out of 107 groups) in the lower right quadrant compared with very few (3 out of 107) in the upper left.

Mean size at release had no detectable impact on straying. Regression of between- and within-catchment straying rates on mean release weight for individual tag groups yielded squared correlation coefficients of 0.005 (N = 120) and 0.028 (N = 107), respectively. Neither coefficient differed significantly from zero at a confidence limit of 95%. There was a weak but

TABLE 4. Incidence of straying between catchments, and within the Rakaia catchment, in relation to age at recovery for 1978–84 brood year Glenariffe chinook salmon. Data shown for each age-class are the total number of tags recovered and the percentage of strays.

Age at recovery	Between catchments		Within catchment	
	Total recoveries	% strays	Total recoveries	% strays
2	2 697	10.7	2 039	12.3
3	10 805	11.3	7 750	16.2
4	4 153	15.2	2 459	18.3
5	16	18.8	6	0.0
Total	17 671	12.3	12 254	16.0

significant correlation (r^2 = 0.057, N = 120) between straying to other catchments and percentage survival to maturity, but this disappeared when three data for July releases with unusually high survival (>7%) were omitted from the calculation. There was no significant correlation between within-catchment straying and percent return (r^2 = 0.028, N = 107).

Age and Sex of Strays

Straying tended to increase with age at maturity (Table 4). Excluding 5-yr-old fish, which were poorly represented, between-catchment straying increased from 10.7% for 2-yr-olds to 15.2% for 4-yr-olds, while within-catchment straying increased from 12.3 to 18.3%. However, interpretation of these differences is confounded by variations in the proportions of recovered tags for each age-class, depending on the mode of recovery. Recoveries from anglers were biased towards older fish. Within the Rakaia catchment, anglers accounted for 15.7, 20.5, and 32.2% of the tags recovered from fish of age 2, 3, and 4, respectively. Since anglers were by far the largest source of tag recoveries from other catchments, the increased straying rates for 4-yr-old fish may have been an artefact of this bias. The increase in within-catchment straying with age is more likely to be real, since only 5 out of 1954 fish positively identified as strays were caught by anglers. To eliminate the possibility that increased straying rates for older fish resulted from biased tag recovery, we examined straying between Glenariffe and two other Rakaia River hatcheries (Coleridge and Blackford), for which we assumed all tags were reported. Straying

rate varied with age ($\chi^2 = 28.9$, df = 2, $p < 0.001$), ranging from 5.0% at age 2 (92 out of 1831 fish) to 7.1% at age 3 (480 out of 6734 fish) to 9.5% at age 4 (195 out of 2058 fish).

Closer inspection of the data for straying within the Rakaia catchment suggested a possible interaction between release date, age at return, and the incidence of straying. To clarify this, we calculated straying rates independently for each age-class, according to the month of release (see Table 3). Straying by 2- and 3-yr-old fish varied with month of release (2-yr-olds: $\chi^2 = 60.6$, df = 9, $p < 0.001$; 3-yr-olds: $\chi^2 = 106.0$, df = 11, $p < 0.001$) but showed no consistent seasonal pattern. By contrast, straying by 4-yr-olds showed a pattern with respect to month of release that was even more extreme than that observed for between-catchment straying. Straying rates amongst 4-yr-old fish ranged from 23.0 to 30.5% for releases between May and August, but averaged only 3.4% for releases from September through to March.

To determine whether straying rates varied between sexes, we analysed data for 4406 tagged chinook recovered at the Glenariffe and Coleridge hatcheries in 1984 and 1985, as this was the only period for which sex data for recovered fish were consistently available for more than one location. To allow for the possibility of bias arising from differential straying rates between age groups and the scarcity of 2-yr-old female fish, we also compared straying rates for individual age groups and then separately analysed brood year 1982, the best represented brood. No differences between straying rates for males and females were detected for the full data set (females 6.4%, males 5.8%; $\chi^2 = 0.88$, $p = 0.35$), for all 3-yr-old fish (females 6.8%, males 8.6%; $N = 2999$, $\chi^2 = 3.45$, $p = 0.06$), or for 3-yr-old fish from the 1982 brood (females 7.9%, males 9.6%; $N = 2577$, $\chi^2 = 2.26$, $p = 0.13$).

Discussion

Any discussion of homing and straying makes implicit assumptions about the spatial scale of a fish population's spawning grounds. In the present context, salmon entering the wrong catchment from the sea have made a much more serious orientation error than those straying within their natal catchment. Within-catchment straying by Glenariffe chinook salmon occurred over a scale of about 50 km, with the majority of strays recovered within the 20 km from Coleridge Hatchery to the Hydra Waters. Over 50% of the strays entered Double Hill Flats Stream, which joins the Rakaia only 1 km above Glenariffe. Such a small spatial scale clearly yields a weaker measure of straying but still indicates some disruption of the homing performance. West and Goode (1987) questioned whether returning salmon could distinguish between areas as small and physically similar as the individual spawning streams in the upper Rakaia catchment. Our results, showing 84% homing to Glenariffe Stream, demonstrate that they can.

Overall straying rates for Glenariffe chinook salmon (12.1% to other catchments and 16.0% within the Rakaia catchment) are generally comparable with, or higher than, those reported for North American chinook populations. Straying rates from several Colombia River hatcheries ranged from 0.8 to 18.1% (Quinn and Fresh 1984; McIsaac and Quinn 1988; Quinn et al. 1991). Straying to other catchments appears to be rare. Combining the data from Quinn and Fresh (1984) and Quinn et al. (1991), only 11 out of an estimated 43 014 tagged chinook were recovered outside the Columbia River drainage (all to the north), representing a between-catchment straying rate of 0.03%. However, distances between the various tributaries of the lower Columbia (which extends 234 km from Bonneville Dam to the sea) are comparable with the distance from the Waiau to the Rangitata, and the mean flows of tributaries (e.g., Cowlitz, 145 $m^3 \cdot s^{-1}$; Lewis, 115 $m^3 \cdot s^{-1}$) are also comparable with those of rivers such as the Waimakariri and Rangitata. In this context, our results for straying between catchments are similar to those for the lower Columbia River tributaries.

The geographical distribution of strays is consistent with the normal range of New Zealand chinook salmon along the east coast of the South Island, generally considered to be from the Wairau to the Clutha, with only isolated specimens found in North Island rivers (McDowall 1990). Most strays were recorded north of the Rakaia. Two smaller northern rivers, the Waiau and Hurunui, which support relatively minor runs of salmon, jointly accounted for 36% of the strays. By contrast, very few Glenariffe fish entered the Waitaki, a large river that (along with the Rakaia, Waimakariri, and Rangitata rivers) supports one of the four major east coast salmon populations, and none penetrated as far south as the Clutha River. The Rangitata was the only river south of the Rakaia to attract a significant number of strays. This may be attributable to the Rangitata Diversion Race, an irrigation and hydroelectric canal that diverts Rangitata water at 30 $m^3 \cdot s^{-1}$ into the Rakaia River via the Highbank power station. For juvenile chinook of Rakaia origin, any olfactory cues acquired below Highbank would be based partly on Rangitata River water. While there is no direct evidence that this diversion of Rangitata water causes Rakaia fish to stray to the Rangitata River, there is evidence that Rangitata fish in the Rakaia River are attracted to the outfall. During a maintenance shutdown of the Highbank power station in 1986, a number of adult salmon were exposed in the dewatering tailrace. Among these were 50 coded-wire-tagged fish, of which 4 (8%) were strays from the Rangitata River. This constitutes a rather high proportion of the fish in the tailrace, as only 14 out of 6100 tags recovered elsewhere in the Rakaia River that year were of Rangitata origin.

Our attempts to model the distribution of strays as a function of distance from the Rakaia River and discharge met with limited success, although the general form of the model seemed appropriate. The best fitting models (which took into account the northward skew in the data) suggested that the number of strays attracted to a particular river increased more or less linearly with discharge (since $\beta \approx 1$) and decreased exponentially with the square root of distance. Other factors specific to each river that could be incorporated into such a model might include the magnitude of the existing spawning population, water temperature, and water chemistry. However, because of the small size of the data set and the limited number of degrees of freedom available, we did not pursue the model further.

The tendency for Glenariffe salmon to stray to the north is consistent with what little is known about their marine distribution and migration patterns. The distribution of juvenile salmon during at least the first few months at sea may be influenced by the Southland current, which flows northeastwards, parallel to the coast (Heath 1985). Virtually all data on the marine distribution of adult salmon have been gathered from incidental catches by domestic trawlers fishing off the Canterbury coast and tend to reflect the distribution of fishing effort (Flain 1981; Unwin et al. 1988a). Based on these data, salmon are widely but thinly distributed in inshore waters along the east coast of the South Island between the Wairau and Clutha rivers. However, a major concentration develops off Banks Peninsula

(midway between the Waimakariri and Rakaia rivers) during December, January, and February. Recoveries of coded-wire-tagged salmon from these catches included significant numbers of Glenariffe fish (e.g. 1017 in 1986–87; Unwin et al. 1988a).

Most adult salmon enter the Rakaia River between January and March (Unwin and Davis 1983). Their transitory appearance off Banks Peninsula a month or two beforehand, together with large numbers of strays in the rivers north of the Peninsula, indicates that salmon initially migrate to the north, with a return migration southwards as they mature. In so doing, their passage takes them by the mouths of the Waiau, Hurunui, and Waimakariri rivers, providing an opportunity for individuals to enter these rivers. The strays in the Rangitata and other rivers to the south could have been salmon that missed the Rakaia and continued southwards. This would be analogous to the situation in the lower Columbia River, where some salmon stray several hundred kilometres upstream, well above Bonneville Dam (Quinn et al. 1991). Alternatively, the strays might have been salmon that migrated south after leaving the Rakaia River.

The most striking characteristic of the Glenariffe data was the relationship between release date and straying. Salmon released during late autumn and winter (May–August) were four times more likely to stray to other catchments than those released in spring and summer (September–February). A similar phenomenon has been reported for Atlantic salmon (*Salmo salar*) (Hansen and Jonsson 1991), although their results were confounded by the release of salmon directly into seawater 4 km from the hatchery. The persistence of the seasonal pattern displayed by Glenariffe chinook, over a period of seven brood years and 10 recovery years, shows that the variation is biologically significant and not merely an artifact of some local and/or short-term environmental effect.

Interpretation of how release data affects straying within and between catchments hinges on the migration patterns of naturally produced juvenile chinook salmon. In Glenariffe Stream, chinook fry emerge from the stream gravels in late winter to midspring (early August to late October; Unwin 1986). About 95% of these fry leave the stream almost immediately, probably within 24 h of emergence, and enter the upper reaches of the Rakaia River. Most of the remaining fish remain in the stream for 3–6 mo, entering the Rakaia during November, December, and January. A small fraction of the stream residents, less than 0.1% of the original cohort, remains within Glenariffe Stream through the winter, finally migrating as yearlings during August–October of the following year. Thus the great majority of chinook salmon must imprint for subsequent homing to Glenariffe Stream within days or at most a few months after emergence, not at the time of smolting and seawater entry.

Within the Rakaia River, fry from Glenariffe and other spawning tributaries begin to accumulate in the upper river during August and disperse downstream, reaching the lower river by October (Hopkins and Unwin 1987). Maximum abundance in the lower river occurs during November–January. Most fish apparently have left the river by March (late summer). Migration patterns of yearling chinook are less well understood. Juveniles from a given cohort have been observed in the tidal lagoon at the Rakaia mouth every month until September of the following year (Eldon and Greager 1983), but the time of year when the majority enter the ocean is not known. However, the scale patterns of Rakaia River adult chinook, comprising both ocean- and stream-type fish (Healey 1983; Flain and Glova 1988; T.P. Quinn and M.J. Unwin, unpubl. data), indicate greatly reduced rates of entry to seawater during winter.

Glenariffe chinook released from the hatchery between March and August migrate downriver at a season that does not correspond to the migratory behaviour of naturally produced salmon. Seining in the lower river during autumn and winter 1985 showed that catches of hatchery fish peaked a few days after release (Unwin et al. 1989), suggesting that the bulk of the population did not remain long in freshwater. Thus the transition from freshwater to seawater also occurred at an atypical time of year. We interpret the failure of many winter-released salmon to return to the Rakaia River as evidence that imprinting to the river, occurring near the transition to seawater, was disrupted.

Among the salmon that homed to the Rakaia River, the proportion homing to Glenariffe Stream showed little relation to the season of release. Presumably, both the hatchery-reared and naturally produced salmon imprinted during incubation or near the time of emergence. The proportion of the population that subsequently strayed from Glenariffe Stream to spawn elsewhere within the Rakaia catchment was less dependent on when they had been released than the proportion that strayed to other catchments. We hypothesise that imprinting occurred at two distinct stages, emergence and transition to sea. Thus the process of homing consisted of first locating the natal catchment, based on odours learned at ocean entry, followed by homing to the natal tributary based on odours learned near emergence. Our data do not reveal any additional seasons or stages during which imprinting occurred, but we cannot rule them out. Sequential imprinting was proposed by Harden Jones (1968) and is supported by the results of transportation experiments explicitly proposed by him (Quinn et al. 1989 and references therein).

The practice of releasing chinook from Glenariffe during autumn and winter evolved in the early 1980s, in response to continuing poor returns from releases of "90-day smolts" during summer (Unwin et al. 1988a). Marine survival of these salmon averaged 0.24% for the 1977–81 broods. Returns to other hatcheries from summer releases were even poorer (Hardy 1988; Unwin et al. 1988a). The 1982–84 time and size at release programme (Unwin et al. 1989) yielded a maximum survival of 7.56% from a winter release of near-yearlings in July 1983 (personal observation). Although wild salmon in the Rakaia River seldom emigrate in winter, the relatively high survival rates of hatchery-produced salmon released in winter are consistent with the fact that seawater tolerance apparently peaks at that time (Franklin et al. 1992). Oddly enough, the seasonal peak in thyroxine (Dickhoff and Sullivan 1987) normally associated with smolt transformation and seawater tolerance (as well as imprinting) was not evident in Glenariffe salmon (Hopkins and Sadler 1987).

We are thus presented with two paradoxes. First, while marine survival and smolting (inferred from seawater tolerance) of hatchery fish were linked, the expected association occurred at a time of year inconsistent with the migratory behaviour of wild fish in New Zealand and of North American populations (Buckman and Ewing 1982; Clarke and Shelbourne 1985; Healey 1991). Second, the association between smolting (again, inferred from seawater tolerance) and olfactory imprinting (inferred from homing to the catchment of origin) was missing. Homing to the Rakaia River was strongest for salmon released at the normal migratory season (summer to early autumn), despite the absence of physiological processes (seawater tolerance and thyroxine peaks) normally associated with downstream migration, smolting, and imprinting (Hasler and Scholz 1983).

The tendency for older salmon to stray more than younger ones is consistent with several other studies of Pacific salmon (Quinn and Fresh 1984; Quinn et al. 1991). Quinn et al. (1991) suggested memory loss in older fish and subtle changes in water chemistry over the years (making rivers and/or hatcheries less recognisable to older fish) as two mechanisms that might be responsible for this phenomenon. However, while older fish were more inclined to stray than younger fish when all release groups were combined, straying rates within the Rakaia catchment for fish released between September and March were actually less for 4-yr-olds (3.4%) than for 3-yr-olds (19.5%) and 2-yr-olds (9.2%). Thus, homing to the Rakaia River was associated with release season irrespective of age at return whereas homing to Glenariffe Stream was associated with release season only for older (i.e., age 4) salmon. It is still not clear why older salmon stray more than younger ones and what physiological and behavioural processes might account for the interaction between release date, age, and straying between and within catchments.

In summary, the results demonstrated considerable variation in the probability that salmon will stray into a given river. The river's discharge, proximity to the natal river, and location (north or south of the Rakaia) all seem to influence the probability of straying. The imprinting by fry to the natal tributary and that by smolts to the mainstem river seem to be largely separate processes, the latter being more strongly influenced by release date than the former. Salmon released in winter showed poorer homing performance to the river of origin than those released in summer, in spite of the higher seawater tolerance and survival of winter releases. Finally, notwithstanding the complex interactions among these factors, older salmon tended to stray more than younger ones, supporting findings for Pacific salmon in other river systems.

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Effect of Tannins on Leaf Processing and Conditioning Rates in Aquatic Ecosystems: An Empirical Approach

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Tannin concentrations were determined in the autumn-shed leaves of 48 species of deciduous trees. Chemical measures of tannins (total phenolics and condensed tannins) were significantly correlated with protein-precipitating capacity. None of these measures of tannin concentration, however, were significantly related to published leaf processing rates, measured as weight loss versus time, or to microbial colonization, measured as rates of lipid synthesis on conditioned leaves. These data suggest that the large variation seen in leaf processing rates is due to factors other than tannin concentration and that much of this variation is due to abiotic factors such as leaching and physical fragmentation. Microbial activity on leaves showed much less variation than did processing rates.

Nous avons mesuré les concentrations de tanins dans les feuilles tombées de 48 espèces d'arbres feuillus. Les mesures des paramètres chimiques des tanins (groupements phénoliques totaux et tanins condensés) marquaient une corrélation significative avec la capacité de précipitation des protéines. Aucune de ces mesures des concentrations de tanins n'était toutefois significativement reliée aux données publiées sur les taux de transformation des feuilles, mesurés en perte de poids en fonction du temps, ni à la colonisation microbienne, mesurée en taux de synthèse des lipides sur les feuilles conditionnées. Ces données semblent indiquer que la forte variation observée dans les taux de transformation des feuilles est liée à des facteurs autres que la concentration de tanins et qu'une bonne partie de cette variation est due à des facteurs abiotiques comme le lessivage et la fragmentation physique. L'activité microbienne sur les feuilles présentait beaucoup moins de variation que les taux de transformation.

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Woodland streams are thought to be heterotrophic systems. Although photosynthetic organisms may be present, the overwhelming source of energy for woodland stream communities is organic matter imported from the adjacent forest community, largely in the form of autumn-shed leaves (Fisher and Likens 1973; Bärlocher 1985). Leaves in streams are colonized by aquatic fungi and bacteria, and this leaf–microbe complex is consumed by a variety of detritivorous stream insects and other arthropods (Kaushik and Hynes 1971). These detritivores are in turn consumed by predators. The apparent paradox, that a food web supported by a seasonal energy pulse is temporally stable, has been explained by the observation that the leaves are consumed very gradually and that while leaves of many species are physically available at the same time, the stream organisms display a distinct sequential preference and generally do not consume much of the less preferred leaves until the supply of the more preferred leaves has been exhausted.

It has been shown that stream detritivores prefer those leaves with the largest biomass of colonizing microorganisms. In fact, it has been hypothesized that the presence of these microorganisms enhances the food value of leaf material; the leaves function only as a substrate to attract and concentrate protein-rich microorganisms (Bärlocher 1985). This hypothesis leads to the suggestion that the apparent preference displayed by the detritivores is in response to differential microbial colonization rates. What factors determine these rate differences?

Many possible factors may affect microbial colonization of autumn-shed leaves. The hypothesis examined here is that microbial access to leaf material is restricted by residual concentrations of chemical defense compounds. This hypothesis was recently tested and tentatively supported by Stout (1989). Here, leaf processing rates defined as $\ln(W_d/W_0)/d$ (Peterson and Cummins 1974) from an extensive compilation by Webster and Benfield (1986) were graphed as a function of relative tannin concentrations compiled from the literature. The positive slopes of the graphs for both temperate and tropical species suggest support for the influence of tannins on processing rates. These intriguing results must be interpreted cautiously. First, the relationship is qualitative, and the strength of the relationship between tannin concentration and processing rate cannot be evaluated. Second, leaf processing rates are calculated on the basis of weight loss, but not all weight loss is biologically mediated. Appreciable mass can be lost readily by leaching processes and over a longer period by abrasion and physical fractionation. Misinterpretation can result if leaves have readily soluble components or are fragile and easily fragmented. They will have high processing rate constants even when they are not processed rapidly by biotic processes. In reality, the processing rate constant $k = k_{\text{physical}} + k_{\text{chemical}} + k_{\text{biological}}$. If we are to test any hypothesis about the effect of residual defenses, we must minimize factors causing weight loss which will be unaffected by these defenses. Third, just how should tannin concentrations be determined? There appears to be little consensus

in the literature, and Martin and Martin (1982) were unable to demonstrate significant correlations between several different measures of tannin concentration.

Three specific hypotheses are examined here. The first is that concentrations of tannins in autumn-shed leaves, measured chemically, correlate well with the potential to precipitate protein, the proposed mode of action (Zucker 1983). The inability of Martin and Martin (1982) to find any correlation may be a result of both small sample size and the use of related leaf species with a narrow range of tannin concentrations. The second is a retest of Stout's (1989) hypothesis using one or more quantitative measures of tannin concentration and the processing rate constants of Webster and Benfield (1986). The third is that some relationship exists between a more direct measure of microbial activity and tannin concentration. This latter hypothesis is an attempt to eliminate the confounding influences inherent in the use of processing rates based on weight loss.

Methods

Leaves were collected immediately prior to abscission from 48 species of deciduous trees in Crawford County, Pennsylvania (41°40′N, 80°W). Most of these species were native riparian trees that could contribute to the total allochthonous loading of temperate streams of the Eastern Deciduous Forest. A number of "exotic" species were included in the collection to increase sample size and to expand the range of measured tannin concentrations. "Exotic" here means tree species not natural to this location but often planted as ornamentals such as *Catalpa* and *Ginkgo*.

Leaves were air-dried and ground to a fine powder. Weighed portions of the ground leaf powder were extracted three times with 50% boiling aqueous methanol. Extracts were pooled for the following analyses: condensed tannins, total phenolics, and protein-precipitating capacity.

Condensed tannins were analysed using the method of Swain and Hillis (1959). Duplicate aliquots (0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 mL) of the alcoholic extracts were made up to 1.0 mL with 50% aqueous methanol, and 8.0 mL of leucoanthocyanin reagent was added. Half of the aliquots were heated in a boiling water bath for 40 min. Optical densities were read in a 1-cm cell at 550 nm, and the differences in optical density between respective heated and unheated samples were graphed as a function of the mass of leaf powder extracted in the aliquot. The slope of this line (optical density per milligram) was taken as a measure of condensed tannin concentration. Martin and Martin (1982) gave a convincing rationale for this approach. Total phenolics were estimated using the Folin–Denis assay (Swain and Hillis 1959; Martin and Martin 1982). Protein-precipitating capacity of the extracts was estimated using the technique of Asquith and Butler (1985). All of these analyses were done in triplicate, and the mean optical density per milligram of leaf powder extracted is reported here.

Leaf processing rates are commonly reported as "k", the slope of the regression of $\ln(W_d/W_0)$ on time (Peterson and Cummins 1974). Webster and Benfield (1988) compiled a list of 596 processing rate coefficients from the literature on 196 plant species. Twenty-six of these species are included in this analysis. I used both calculated mean and median values of k for each species examined.

As a measure of microbial activity on leaves, I estimated the rates of microbial lipid synthesis on conditioned leaves as proposed by White et al. (1977). To measure activity on leaves, leaf disks were punched from each of the 48 species with a 12-mm-diameter cork borer and placed in 4-L culture dishes with stream water collected from Sandy Run, Crawford Co., PA. Stream water was renewed on alternate days to insure adequate bacterial and fungal inocula and to dilute accumulated wastes and leachate. After a 9-d conditioning period at 10°C, leaf disks and attached microflora were transferred to 2.0 mL of distilled water containing 37 kBq of ^{14}C-labeled acetate. Disks were incubated for 2 h and then activity was stopped by transferring the disk to a MeOH, chloroform, water, and HCl solution. Extraction of lipids followed White et al. (1977), and activity was determined by liquid scintillation. This experiment was repeated six times using a different leaf for each disk cut. Total activity as lipids was analysed initially using a randomized block ANOVA (trial × species) and later using mean rank order of activities.

Further analysis used correlation or regression on the means or natural log transformed means of replicate data.

Results and Discussion

Total phenolics spanned a range from 0.055 to 1.849 optical density units (OD)/mg (Table 1). The collection of leaves examined also showed a broad range in condensed tannin concentration (0.003–0.276 OD/mg) and protein-precipitating capacity (0.005–0.248 OD/mg) (Table 1).

The first hypothesis to be tested was that there exists a relationship between the concentration of tannins in autumn-shed leaves and protein-precipitating capacity. Martin and Martin (1982) were unable to show any correlation between condensed tannin, total phenolics, and protein-precipitating capacity in six species of *Quercus*. One possible reason might be that the ranges of values of total phenolics, condensed tannin, and protein-precipitating capacity were small among a few species in one genus. Empirical relationships in ecology are more apparent when data values span at least an order of magnitude. I found highly significant correlations between ln protein-precipitating capacity and both ln total phenolics ($r = 0.630$, $p < 0.001$) (Fig. 1a) and ln condensed tannins ($r = 0.453$, $p < 0.01$) (Fig. 1b) using the 48 species with a much larger range in measured values for each parameter. Since the measured values for these parameters were not normally distributed, correlations were calculated using natural log transformed data.

Having established that total phenolics and condensed tannins are related to protein precipitating capacity, it seemed reasonable to assume that fungi and bacteria colonizing leaf tissue might be deterred by high concentrations of these tannins (Zucker 1983). The work of Stout (1989) suggests a possible correlation between leaf processing rate, k, and relative tannin concentration. Using the same k values compiled by Webster and Benfield (1986) and the results of the analyses present in Table 1, I found no significant correlation between either mean or median k and protein-precipitating capacity, total phenolics, or condensed tannins (Fig. 2). Again, since median and mean k values were not normally distributed, correlations were calculated using natural log transformed data.

One possible reason for the lack of correlation between leaf processing rate and any measure of tannin concentration might be that k is influenced by a variety of abiotic processes. I make the assumption that residual concentrations of tannins affect biotic processing only. Consequently, I used a more direct measure of microbial processing of leaves, the rate of lipid synthesis on conditioned leaves (White et al. 1977). Presumably,

TABLE 1. Mean concentrations (OD/mg leaf tissue) of total phenolics (TP), condensed tannins (CT), and protein-precipitating capacity (PPC), and mean rank of ^{14}C-labeled lipid activity of the 48 leaf species examined. Taxonomic nomenclature follows Gleason and Cronquist (1963). Raw data are available on request.

Family and species	TP	CT	PPC	Lipid rank
Aceraceae				
Acer negundo	0.220	0.022	0.006	11.17
Acer saccharum	1.337	0.128	0.037	13.00
Acer rubrum	1.681	0.101	0.014	22.50
Acer saccharinum	1.432	0.015	0.007	27.17
Acer platanoides	0.738	0.083	0.012	14.83
Anacardiaceae				
Rhus typhina	1.849	0.041	0.248	37.33
Betulaceae				
Betula lutea	0.364	0.042	0.007	10.17
Betula populifolia	0.707	0.101	0.010	22.33
Carpinus caroliniana	1.072	0.024	0.011	16.67
Alnus rugosa	1.006	0.036	0.008	22.00
Bignoniaceae				
Catalpa speciosa	0.055	0.003	0.005	28.67
Caesalpiniaceae				
Cercis canadensis	0.361	0.014	0.006	14.83
Gleditsia triacanthos	0.858	0.147	0.015	41.33
Cornaceae				
Cornus florida	1.326	0.112	0.177	21.67
Cornus stolonifera	1.044	0.053	0.017	14.50
Nyssa sylvatica	1.079	0.006	0.032	28.83
Fabaceae				
Robina pseudoacacia	0.658	0.224	0.061	26.83
Fagaceae				
Fagus grandifolia	0.212	0.043	0.007	12.83
Quercus palustris	0.824	0.045	0.022	29.33
Quercus coccinea	0.983	0.017	0.030	10.17
Quercus muehlenbergii	0.654	0.033	0.012	28.83
Quercus alba	0.946	0.039	0.038	24.33
Quercus prinus	0.915	0.107	0.056	31.50
Quercus rubra	0.598	0.060	0.012	26.83
Hamamelidaceae				
Hamamelis virginiana	0.863	0.051	0.018	15.33
Liquidambar styraciflua	0.800	0.044	0.028	23.17
Hippocastanaceae				
Aesculus hippocastanum	0.502	0.104	0.010	23.83
Juglandaceae				
Juglans nigra	0.525	0.011	0.013	39.33
Juglans cinerea	0.667	0.115	0.014	39.33
Carya glabra	0.311	0.025	0.008	31.00
Carya laciniosa	0.212	0.033	0.008	19.33
Carya ovata	0.298	0.028	0.008	30.17
Lauraceae				
Sassafras albidum	0.298	0.053	0.006	19.20
Magnoliaceae				
Liriodendron tulipifera	0.420	0.024	0.014	24.00
Magnolia acuminata	0.267	0.004	0.005	38.00
Oleaceae				
Fraxinus americana	0.256	0.004	0.006	36.17
Platanaceae				
Platanus occidentalis	0.613	0.138	0.019	14.83

TABLE 1. (*Concluded*)

Family and species	TP	CT	PPC	Lipid rank
Rosaceae				
Crataegus sp.	0.607	0.135	0.013	19.50
Pyrus malus	0.624	0.039	0.011	23.83
Prunus serotina	0.744	0.138	0.012	16.17
Salicaceae				
Salix discolor	0.934	0.239	0.080	27.83
Salix nigra	0.631	0.100	0.010	31.00
Populus deltoides	0.613	0.101	0.012	21.67
Populus tremuloides	0.767	0.138	0.016	25.17
Populus grandidentata	1.082	0.276	0.131	12.67
Tiliaceae				
Tilia americana	0.310	0.076	0.023	28.80
Ulmaceae				
Ulmus sp.	0.893	0.192	0.020	29.33
Ginkgoaceae (Gymnospermae)				
Ginkgo biloba	0.429	0.120	0.011	40.67

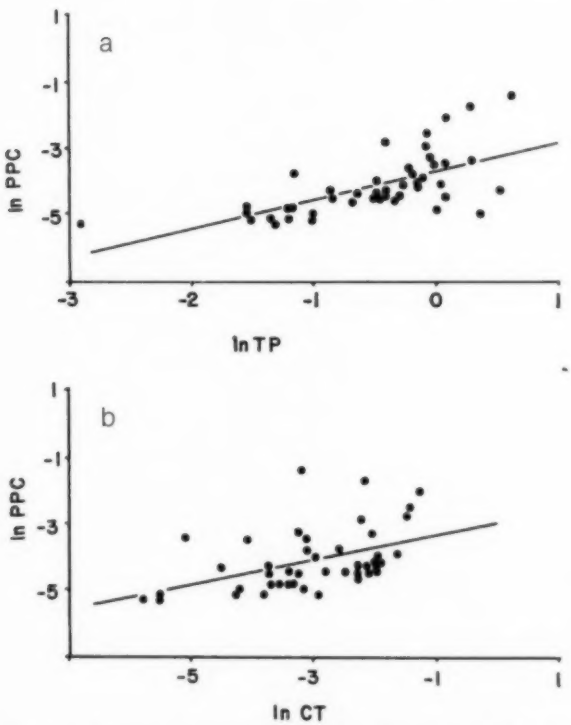

FIG. 1. Relationship between (a) natural log transformed total phenolics (ln TP) and (b) natural log transformed condensed tannins (ln CT) and protein-precipitating capacity (ln PPC) in the leaves of 48 species of deciduous trees. Regression equations: Fig. 1a, $\ln PPC = 0.87 \ln TP - 3.70$, $n = 48$, $r^2 = 0.40$, $p < 0.001$; Fig. 1b, $\ln PPC = 0.38 \ln CT - 3.00$, $n = 48$, $r^2 = 0.21$.

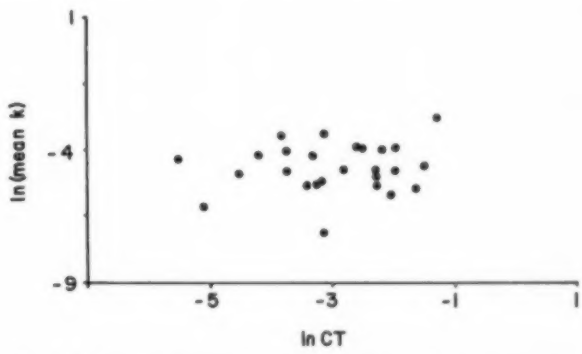

FIG. 2. Relationship between natural log transformed mean processing rates (ln k) and natural log transformed condensed tannin concentrations (ln CT) ($n = 26$, $r = 0.16$, $p > 0.05$).

the rate of lipid synthesis is a reasonable analog for microbial biomass or activity, and leaves that are readily colonized by bacteria and fungi would support higher rates of lipid synthesis. Lipid activity on leaf disks was highly variable as anticipated (White et al. 1977), and the randomized block ANOVA indicated that the results of the six trials were different, perhaps as a result of daily variations in inoculum or stream water quality. Rather than pool the lipid activities from each trial and calculate a mean activity by species, I rank ordered these activities and calculated a mean rank (Table 1). The lowest ranked species (showing the least ^{14}C-labeled lipid activity) were *Quercus coccinea* and *Betula lutea*. The highest ranked species (most activity) were *Gleditsia triacanthos* and *Ginkgo biloba*. There appeared to be some pattern in the rankings; species in the family Fagaceae, for example, tended to have low rankings, and species in the family Juglandaceae generally had high rankings. These data are consistent with the general observation that hickory leaves are processed faster than oak leaves in streams (Kaushik and Hynes 1971). However, I found no significant correlation between mean rank and either condensed tannin, total phenolics, or protein-precipitating capacity. Nor was there any significant correlation between mean rank and mean or median k.

It is reassuring to find that chemical measures of tannins in leaves (proanthocyanidin and Folin–Denis assays) are correlated with at least one biological measure (protein-precipitating

capacity). These correlations suggest that the deterrent value of tannins may be inferred from the relatively simple chemical assays which also tend to have lower variances. Tannins, however, are not correlated with either leaf processing rates or microbial colonization rates in aquatic systems. There are at least several possible hypotheses that may explain this lack of correlation. The obvious one is that perhaps there is no effect of tannins on microbial activity and resultant decomposition rates, and the rate differences we see are due to species differences in nitrogen content, C:N ratios, toughness, etc., or these are contributing factors that mask the effects of tannins alone. Attempts to alter the nutrient content of leaves have been confounded by other factors (cf. Irons et al. 1988), although experiments where nutrients are added to the water generally show increased decomposition rates (Meyer and Johnson 1983). Another possibility is that the tannin content of leaves falling into streams and lakes might be quickly reduced by leaching and that microbial access is uninhibited after only a short time when other factors come into play (Valiela et al. 1979). Yet another possibility is that despite White et al.'s (1977) convincing support, microbial lipid synthesis is not a reasonable measure of microbial activity.

Published processing rates of leaves falling into streams span over three orders of magnitude (Webster and Benfield 1988) and, in the collection of leaves examined here, span almost two orders of magnitude. Leaf tannin concentrations do not explain any significant amount of the variation in these rates. The range of values in lipid synthesis rates, however, varied by less than a factor of 3 (mean 2.9; SD 0.6, based on six experiments). Here again, tannins failed to explain any significant amount of variation. It is evident that much of the variation in processing rates we see is due to factors not directly related to microbial activity and that these other factors might confound attempts to test alternative hypotheses (e.g., internal nutrient concentrations, C:N ratios, lignins, etc.) explaining variations in processing rate. Leaf processing defined as weight loss is an important measure of the rate at which coarse detritus is converted to dissolved and fine particulate organic matter and thereby made available to other components of aquatic food webs. It should be recognized, however, that a significant portion of this weight loss is not biologically mediated and therefore will be independent of factors that might have an influence on the biotic activity of fungi and bacteria on leaf surfaces.

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Estimating Salmon Spawning Escapement Using Capture–Recapture Methods

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We describe a method of estimating the spawning escapement of coho salmon (*Oncorhynchus kisutch*) from capture–recapture data. Traditional capture–recapture analyses do not directly provide estimates of escapements; however, we show how simple modifications to the Jolly–Seber method can estimate the total number of fish returning to a river including those that enter and die between sampling occasions. Spawning runs of Pacific salmon were simulated and their escapements estimated using capture–recapture. The performance of the maximum likelihood estimators (MLEs), the censored MLEs, the constrained MLEs, and less-biased estimators in estimating the run sizes and providing estimates of precision were evaluated. Simulation results indicated that constrained MLEs provided the most appropriate estimates of escapement and that standard errors be computed using the large-sample variance formulae evaluated at these estimates. These methods were used to estimate the escapements of coho salmon to a small river on Vancouver Island in 1989 and 1990.

Nous décrivons une méthode permettant d'estimer les échappées de géniteurs de saumons coho (*Oncorhynchus kisutch*) à partir de données sur les prises et recaptures. Les analyses traditionnelles de ce type ne donnent pas des estimations directes des échappées; nous montrons toutefois comment des modifications simples apportées à la méthode de Jolly–Seber peuvent permettre d'estimer le nombre total de poissons remontant dans un cours d'eau, y compris ceux qui entrent et meurent entre les échantillonnages. Les remontes de saumon du Pacifique ont été simulées, et leurs échappées estimées par prises et recaptures. Nous avons évalué la performance des estimateurs de vraisemblance maximale (MLE), des MLE tronqués, des MLE contraints et d'autres estimateurs moins biaisés pour calculer la taille des remontes et donner des estimations de la précision. Les résultats de la simulation indiquent que les MLE contraints ont donné les estimations les plus appropriées des échappées, les erreurs types étant calculées à l'aide des formules de variance de grand échantillon déduites de ces estimations. Ces méthodes ont servi à estimer les échappées de saumon coho dans une petite rivière de l'île de Vancouver en 1989 et 1990.

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1. Introduction

The need for better salmon spawning escapement estimates, and the standardization of estimation procedures, has been well documented (Walters and Ludwig 1981; Pearse 1982; Symons and Waldichuk 1984). Accurate estimates of spawning stocks are required to assess whether stock sizes and harvest rates are at an optimum, and precise estimates of escapement are required if fishery managers are to have confidence in management strategies. These objectives may be achieved in various ways. A counting fence or weir has traditionally been the most reliable method of escapement enumeration, although fences are prone to flood damage and costly to operate. Recent developments in visual survey techniques (Tschaplinski and Hyatt 1990; Irvine et al. 1992) have also resulted in acceptable estimates of escapement. In this paper, we describe a method to estimate the total number of coho salmon (*Oncorhynchus kisutch*) returning to a stream to spawn using capture–recapture methods.

Previous capture–recapture studies on Pacific salmon have focused on the use of closed models or the Petersen estimator. Closed models are appropriate for populations that have no recruitment or losses during the study. The Petersen estimate has been employed for many salmon stocks, and its use for escapement was reviewed by Cousens et al. (1982) and Simpson (1984). Because recruitment and losses can occur during the course of a study, attention has moved to models for open populations, and Labelle (1990) developed an iterative approach "open model" for estimating salmon escapements.

The Jolly–Seber model (Pollock et al. 1990) is an appropriate tool for estimating abundances of open populations in capture–recapture studies. However, it has rarely been used for Pacific salmon, perhaps in part because the analysis of capture–recapture data using the Jolly–Seber model does not provide a

direct estimate of escapement. The Jolly–Seber model does provide estimates of the probability of survival between sampling occasions, of abundance at sampling occasions, and of the net number of new animals that enter between sampling occasions and survive to the next sampling occasion. For escapement estimation, these parameters are of secondary importance. Interest lies in estimating the number of fish present at any time between the first and last sampling occasion including those fish that enter the system and die between consecutive sampling occasions. In addition, the population size of interest includes those fish that entered before the first sampling occasion and those that entered after the last sampling occasion. In a well-designed study, these numbers should be small enough that they can be ignored.

Previous authors have estimated the equivalent of escapement using capture–recapture methods. Crosbie and Manly (1985) modeled the recruitment and survival processes by probability distributions requiring fewer parameters than required by the Jolly–Seber model. Furthermore, they showed that when uniform recruitment and mortality rates are assumed between sampling occasions, the estimates of escapement are simple functions of the Jolly–Seber estimates, and they determined analytic large-sample estimates of precision. McKay and Bradley (1988) followed a similar approach using a different set of distributions than Crosby and Manly for recruitment and survival and used numerical methods to estimate recruitment and survival parameters and their standard errors. Sykes and Botsford (1986) examined the problem of estimating the spawning escapement of chinook salmon (*O. tshawytscha*) by tagging and recovering carcasses. They also modified the Jolly–Seber estimates by assuming that all recruitment took place halfway between sampling occasions and used a parametric bootstrap to estimate the precision of the estimates.

However, several issues that may greatly affect estimates of escapement and their precision remain unresolved. The objectives of this paper are to explore the consequences of these issues and to make recommendations for their resolution. In particular:

(1) What are the effects of different assumptions about recruitment upon estimates of the escapement?

(2) How should recruitment be estimated at the start and at the end of an experiment, when the parameters of interest are confounded?

(3) Should maximum likelihood estimates, which are known to be biased, or less-biased estimators (Pollock et al. 1990) be used? It may occur that the closed-form estimates are outside the parameter space (e.g., estimated births less than zero). How should these be adjusted?

(4) How are estimates of precision obtained? How closely do the asymptotic formulae for the variances and covariances approximate the true sampling error? Is the distribution of the estimates sufficiently close to a normal distribution that the usual large sample confidence intervals may be used with assurance that they are valid?

(5) What are the effects of violations of the assumptions of homogeneous survival and recapture?

Because the Jolly–Seber model forms the basis for our method, we begin by briefly reviewing it and its shortcomings for escapement estimation. Next we propose an estimator for the escapement and then discuss the resolution of objectives 1–4. A simulation example is presented to illustrate the properties of our estimator and to investigate violations of assumptions (objective 5). Finally, 2 yr of experimental data are analyzed.

TABLE 1. Notation.

	Fundamental Jolly–Seber parameters
s	Number of sampling occasions
p_i	Probability of capture at sampling occasion i, $i = 1, \ldots, s$
ϕ_i	Probability that an animal alive at sampling occasion i will be alive at sampling occasion $i + 1$, $i = 1, \ldots, s - 1$
B_i	Number of animals that enter the river system after sampling occasion i and *are still alive* at sampling occasion $i + 1$, $i = 0, \ldots, s - 1$. These are subsequently referred to as "births." B_0 is defined as the number of animals that enter before the first sampling occasion and are alive at the first sampling occasion
	Derived Jolly–Seber parameters
N_i	Number of animals alive in the system at sampling occasion i, i.e., the abundance at occasion i, $i = 1, \ldots, s$. $N_1 = B_0$; $N_{i+1} = (N_i - n_i + R_i)\,\phi_i + B_i$, $i = 1, \ldots, s - 1$
M_i	Number of marked animals alive in the system at sampling occasion i, $i = 1, \ldots, s$
	New parameters
B_i^*	Number of animals that enter the system after sampling occasion i but before sampling occasion $i + 1$, $i = 0, \ldots, s - 1$. $B_i^* \geq B_i$ because it includes those animals that enter after sampling occasion i but die before sampling occasion $i + 1$
N	Total number of animals that enter the system before the last sampling occasion, i.e., the escapement. $N = B_0^* + B_1^* + \ldots + B_{s-1}^*$
	Statistics
m_i	Number of marked animals captured in the ith sample, $i = 1, \ldots, s$
u_i	Number of unmarked animals captured in the ith sample, $i = 1, \ldots, s$
n_i	Number of animals captured in the ith sample, $i = 1, \ldots, s$. $n_i = m_i + u_i$
R_i	Number of the n_i that are released after the ith sample, $i = 1, \ldots, s - 1$. $R_i \leq n_i$ because of losses on capture
r_i	Number of R_i released at i that are captured again, $i = 1, \ldots, s - 1$
z_i	Number of animals captured before i, not captured at i, and captured later, $i = 1, \ldots, s - 1$

2. Jolly–Seber Analysis

Details of the design, required assumptions, and subsequent analysis of capture–recapture experiments using the Jolly–Seber model are presented in Seber (1982) and Pollock et al. (1990). The notation used is shown in Table 1. At each of s sampling occasions, animals are captured. Unmarked animals are individually marked and subsequently released. Tag numbers of previously marked animals are recorded and the animals are also released. The number of releases may be less than the number of captures due to losses on capture or, less commonly, more than the number of captures due to additions by the researcher. There are a total of $3s - 1$ parameters: $p_1, \ldots, p_s$, $\phi_1, \ldots, \phi_{s-1}$, and $B_0, \ldots, B_{s-1}$. The parameters $\{N_i\}$ and $\{M_i\}$ can be derived from these parameters.

A likelihood equation can be constructed that gives the probability of observing the statistics $\{m_i\}$, $\{u_i\}$, $\{R_i\}$, $\{r_i\}$, and $\{z_i\}$

TABLE 2. Closed-form MLEs and less-biased estimators for the Jolly–Seber model.

Parameter	Closed-form MLE	Less-biased estimator	Range
	Unconfounded parameters		
M_i	$\hat{M}_i = m_i + \frac{R_i z_i}{r_i}$	$\tilde{M}_i = m_i + \frac{(R_{i+1})z_i}{(r_{i+1})}$	$2, \ldots, s-1$
N_i	$\hat{N}_i = \frac{n_i \hat{M}_i}{m_i}$	$\tilde{N}_i = \frac{(n_i+1)\tilde{M}_i}{(m_i+1)}$	$2, \ldots, s-1$
p_i	$\hat{p}_i = \frac{m_i}{\hat{M}_i}$	$\tilde{p}_i = \frac{m_i}{\tilde{M}_i}$	$2, \ldots, s-1$
ϕ_i	$\hat{\phi}_i = \frac{\hat{M}_{i+1}}{\hat{M}_i - m_i + R_i}$	$\tilde{\phi}_i = \frac{\tilde{M}_{i+1}}{\tilde{M}_i - m_i + R_i}$	$1, \ldots, s-2$
B_i	$\hat{B}_i = \hat{N}_{i+1} - \hat{\phi}_i(\hat{N}_i - n_i + R_i)$	$\tilde{B}_i = \tilde{N}_{i+1} - \tilde{\phi}_i(\tilde{N}_i - n_i + R_i)$	$2, \ldots, s-2$
	Confounded parameters		
$\phi_{s-1}p_s$	$\widehat{\phi_{s-1}p_s} = \frac{r_{s-1}}{R_{s-1}}$	Same as MLE	
$B_0 p_1$	$\widehat{B_0 p_1} = n_1$	Same as MLE	
$B_1 + B_0(1-p_1)\phi_1$	$\hat{N}_2 - \hat{\phi}_1 R_1$	$\tilde{N}_2 - \tilde{\phi}_1 R_1$	
B_{s-1}/ϕ_{s-1}	$\frac{n_s}{\widehat{\phi_{s-1}p_s}} - (\hat{N}_{s-1} - n_{s-1} + R_{s-1})$	$\frac{n_s}{\widetilde{\phi_{s-1}p_s}} - (\tilde{N}_{s-1} - n_{s-1} + R_{s-1})$	

given these parameters (Seber 1982, p. 198). Closed-form maximum likelihood estimators (MLEs) can be found (Pollock et al. 1990) and are shown in Table 2.

Because escapement is defined as the number of fish that escape marine fisheries and return to a river to spawn, one possible estimator for the escapement could be formed as $\hat{B}_0 + \hat{B}_1 + \ldots + \hat{B}_{s-1}$ which is the sum of the net number of fish that enter the river system between sampling occasions. However, there are several problems with this approach that will be outlined in the remainder of this section and resolved in the next section.

First, the Jolly–Seber estimators of birth estimate the number of animals that enter the population after a sampling occasion *and are alive at the next sampling occasion*. Such estimates exclude those animals that enter the system but die *before* the next sampling occasion which also form part of the escapement. Consequently, Jolly–Seber estimates of birth underestimate the true number of new animals, except when all animals are guaranteed to survive from their entry to the next sampling occasion.

Second, not all of the parameters can be estimated. Only $3s - 7$ parameters of the $3s - 1$ parameters are individually estimable as shown in Table 2. The parameters p_1, p_s, ϕ_{s-1}, B_0, B_1, and B_{s-1} cannot be individually estimated; they are confounded. Four estimable functions of these parameters are shown in Table 2. Consequently, it is impossible to estimate the number of recruits at the start and end of the experiment.

Third, despite the optimal large-sample properties of the MLEs, they are biased when used with small samples (Pollock et al. 1990). This bias occurs in small samples because there is an appreciable chance that m_i will equal zero at some sampling occasion and then the MLE for the number of births will take the value of infinity. The less-biased estimators (Table 2) do not suffer from this defect, but are still biased for small values of the sample statistics. Pollock et al. (1990, p. 21) recommend that m_i and r_i should be at least 10 to obtain satisfactory performance from the less-biased estimators. This may be unobtainable in many studies.

Lastly, it is possible that the estimators in Table 2 may produce estimates that are out of range, e.g., estimates of survival greater than 1 or estimates of births less than zero. Simply censoring the estimates to the boundary value, e.g., setting negative estimates of birth to zero, will result in bias. It is not clear how to modify the likelihood equations to impose the constraints to keep all estimates in bounds.

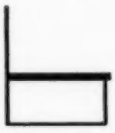
3. Estimating Escapement

3.1. Accounting for Deaths before the Next Sampling Occasion

Two approaches have been proposed to account for deaths of newly recruited fish between sampling occasions.

In the first approach, the recruitment process over the entire experiment is modeled by a probability distribution. Because, by definition, probability distributions must integrate to one over the entire experiment, the fate of all animals will be modeled. Crosbie and Manly (1985) assumed that time of recruitment followed a beta distribution and McKay and Bradley (1988) assumed an Erlangian distribution. An additional advantage of using a probability distribution is that estimates of recruitment at the start and the end of the study are no longer confounded. The disadvantages of this approach are the mathematical complexity and, more seriously, the substantial bias introduced if the mathematical model chosen is inappropriate. Additionally, it may be difficult to find an appropriate probability distribution for bi- or trimodal

TABLE 3. Comparison of the adjustment factors under various distributions of recruitment between sampling occasions (uniform survival rate assumed). Each graph depicts recruitment versus time. The formula under the graph is the adjustment factor as derived in Appendix A.

	Distribution of recruitment between sampling occasions and derived adjustment factors					
	All take place after previous sample time	Declining over time	All take place at midpoint (Sykes and Botsford 1986)	Uniform (Crosbie and Manly 1985)	Increasing over time	All take place at just before next sample time
						
Survival rate between sampling occasions ϕ	$1/\phi$	$\frac{\log(\phi)^2}{2+2\phi\log(\phi)-2\phi}$	$\frac{1}{\sqrt{\phi}}$	$\frac{\log(\phi)}{\phi-1}$	$\frac{\log(\phi)^2}{2\phi-2\log(\phi)-2}$	1
1.00	1.00	1.00	1.00	1.00	1.00	1.00
0.90	1.11	1.07	1.05	1.05	1.04	1.00
0.80	1.25	1.16	1.12	1.12	1.08	1.00
0.70	1.43	1.26	1.20	1.19	1.12	1.00
0.60	1.67	1.40	1.29	1.28	1.18	1.00
0.50	2.00	1.57	1.41	1.39	1.24	1.00
0.40	2.50	1.80	1.58	1.53	1.33	1.00
0.30	3.33	2.14	1.83	1.72	1.44	1.00
0.20	5.00	2.71	2.24	2.01	1.60	1.00
0.10	10.00	3.96	3.16	2.56	1.89	1.00
0.05	20.00	5.61	4.47	3.15	2.19	1.00

distributions of recruitment such as those that occurred in the examples later in this paper.

In the second approach which we prefer, assumptions are made to describe the recruitment process between individual sampling occasions. The likelihood function of the Jolly–Seber model is then modified to incorporate a description of the recruitment process, e.g., Crosbie and Manly (1985) derived a likelihood equation (their equation (6)) suitable when recruitment and mortality are assumed to occur uniformly between sampling occasions. The solution of their likelihood equations yielded identical MLEs for $\{\phi_i\}$, $\{p_i\}$, and $\{B_i\}$ as shown in Table 2. The MLE for the total recruitment between sampling occasions (including those that die before the next sampling time) is

$$(1)\quad \hat{B}_i^* = \hat{B}_i \frac{\log\hat{\phi}_i}{\hat{\phi}_i - 1},$$

i.e., the total number of new recruits is a simple function of the net number of recruits and the survival rate for that period. Crosbie and Manly (1985) ignored losses on capture, but modifications to their results to account for such losses are straightforward (Appendix C).

The assumption of uniform recruitment and mortality rates between sampling occasions cannot be verified with the data collected in a capture–recapture experiment. However, the sensitivity of the adjustment process to the choice of recruitment distributions can be investigated by choosing different patterns for recruitment (Appendix A). The adjustment factors corresponding to various recruitment distributions and mortality rates to be used with the Jolly–Seber estimates of birth (e.g., equation (1)) are shown in Table 3. The adjustment factors are similar when survival rates are high, since most animals that enter between sampling occasions survive to the next sampling occasion. However, the adjustment factors differ considerably for mid- to low survival rates. In such cases, careful consideration must be given to the assumptions about the recruitment process to avoid extreme over- or underestimation of the total recruitment. The adjustment factors in Table 3 can be estimated by replacing the parameters in the formulae by estimators from the Jolly–Seber analysis.

3.2. Accounting for Confounding at the Start and End of the Run

If all recruitment parameters were estimable, then the escapement could be estimated as

$$\hat{N} = \hat{B}_0^* + \ldots + \hat{B}_{s-2}^* + \hat{B}_{s-1}^*$$

where $\hat{B}_i^*$ are obtained from the Jolly–Seber estimates and the appropriate adjustment factor as in equation (1). Because B_0, B_1, and B_{s-1} are not identifiable without further assumptions, Seber (1982, p. 201) suggested on one hand that p_1 and p_s be estimated in an ad hoc fashion from the pattern of recapture rates found in the data. Then the confounded parameters may be individually resolved and the recruitment in the first and last sampling periods estimated. However, any assumptions about p_1 and p_s cannot be tested using information in the model. On the other hand, it may be reasonable on biological and logistical grounds to assume that the capture rates at all sampling occasions are equal, i.e., $p_1 = p_2 = \ldots = p_s$. Under this assumption, all recruitment parameters may be estimated, but closed-form estimators do not exist and numerical methods must be used (Jolly 1982; Pollock et al. 1990, section 5.3.3).

Because it is relatively easy to extend capture–recapture escapement studies until recruitment has virtually ended, we propose to ignore the problem of estimating B_{s-1} and will set it to zero. However, it may be more difficult to start an escape-

ment enumeration study before much escapement occurs. We propose to resolve the problem of confounded parameters at the start of the experiment by applying an adjustment factor to $N_2 = B_0\phi_1 + B_1$, the number of animals alive at the second sampling occasion. Then, for example, under the assumption of uniform recruitment between all sampling occasions, our proposed estimator for the escapement is

$$(2)\quad \hat{N} = \hat{N}_2 \frac{\log\hat{\phi}_1}{\hat{\phi}_1 - 1} + \hat{B}_2 \frac{\log\hat{\phi}_2}{\hat{\phi}_2 - 1} + \ldots + \hat{B}_{s-2} \frac{\log\hat{\phi}_{s-2}}{\hat{\phi}_{s-1} - 1}.$$

Our estimator is slightly biased if all assumptions are valid, since it estimates

$$B_0\phi_1 \frac{\log\phi_1}{\phi_1 - 1} + B_1^* + B_2^* + \ldots B_{s-2}^*$$

which is less than the escapement (ϕ_1 ($\log\phi_1/(\phi_1 - 1)$) is less than 1 and B_{s-1}^* is missing). However, in a well-designed study, there should be little recruitment before the start of sampling or near the end of sampling and the bias is expected to be small.

3.3. Adjusting the Estimates for Small Sample Biases

As noted earlier, the individual MLEs of births are biased upwards (Pollock et al. 1990, p. 20). The less-biased estimators (Table 2) can be used, but Seber (1982, p. 204) indicated that both m_i and r_i should be at least 10 for satisfactory improvements. In studies where m_i and r_i are less than 10, we recommend that adjacent sample occasions be pooled. Arnason and Baniuk (1978, section 5.3) and Hargrove and Borland (1993) indicated that pooling leads to nearly unbiased estimates of the net number of births and survival rates for the pooled period if the mortality rate is low (less than 50%) for each of the pooled intervals. However, pooling may introduce additional bias if the patterns of recruitment are very different in the two intervals, i.e., bias will be introduced if the assumption of uniform recruitment over the pooled intervals is imposed on two nonuniform recruitment patterns in each interval. If pooling is performed, the choice of recruitment distribution for the pooled interval should be given careful consideration.

3.4. Adjusting Out-of-Range Estimates

It is possible that some of the closed-form estimators produce estimates that are out of range, e.g., survival estimates greater than 1 or birth estimates less than zero. This is not unusual and does not indicate that the model is wrong. For example, estimates of net births are essentially found as $\hat{B}_i = \hat{N}_{i+1} - \hat{\phi}_i\hat{N}_i$. Both $\hat{N}_{i+1}$ and $\hat{N}_i$ are highly variable when the capture rate is small (Pollock et al. 1990, p. 72), and consequently, negative estimates of net births can easily occur if the actual number of net births is small. One common solution is to censor the out-of-range estimators to the boundary values, e.g., estimates of survival greater than 1 are set to 1 or estimates of births less than zero are set to zero. However, this can introduce substantial positive bias into the birth estimates (Appendix B). We recommend either the use of numerical methods that constrain all estimates in bounds (constrained MLEs, Appendix C) or the use of the negative estimates as is. The first solution may involve difficult numerical computations. However, when numerical methods force the negative estimate to zero, they simultaneously reduce neighboring estimates so that the sum and therefore the estimated escapement is little changed. The latter option works well because estimates of recruitment in neighboring occasions are highly correlated.

3.5. Estimates of Precision

Two methods used to obtain estimates of the precision of estimators are large-sample asymptotic formulae and bootstrap methods.

Asymptotic large sample variances and covariances were derived for the net recruitment estimates (Appendix D). These are similar to those of Crosbie and Manly (1985) except Crosbie and Manly used a different subscript notation, they did not allow for losses on capture, and they did not use our suggested estimator for the recruitment at the start of the experiment. Our formulae were found conditional upon the escapement rather than upon the actual (unknown) number of births in each interval which explains the differences from the formulae presented in Pollock et al. (1990). Our formulae can than be used in a Taylor-series expansion with the particular recruitment adjustment factor employed and the expansions summed to obtain the variance of the escapement estimate.

Problems can occur in evaluating the asymptotic formulae when estimates are outside the parameter space, e.g., negative estimates of birth. Simply evaluating the formulae using these invalid estimates may lead to nonsensical estimates, e.g., estimated variances less than zero. In this case, numerical methods should first be used to find estimates that are within the parameter space. Then the asymptotic formulae can be evaluated at these constrained estimates after using the constrained estimates to evaluate $E[m_i]$ and other expected statistics that appear in the asymptotic formulae rather than using m_i and other statistics from the experiment. Alternatively, estimated variances can be obtained directly by inverting the information matrix at the last iteration of the constrained numerical optimization. However, the numerical methods used to constrain estimates cannot distinguish between a constraint necessary to keep an estimate within a parameter space and a constraint that represents real knowledge about a parameter and may report estimated variances of zero for such estimates. For example, estimates of births that are constrained to be zero are reported with an estimated variance of zero even though the constraint does not imply that we know that births are zero. Consequently, the standard errors (SEs) obtained from using the inverse of the information matrix following a constrained maximum likelihood estimation may be underestimates of the true SE, particularly if many parameters were constrained.

A confidence interval for the escapement formed by multiplying the estimated SE by a confidence factor and bracketing the estimate (e.g., estimate $\pm$ 1.96 SE) is expected to perform well even though the usual Jolly–Seber estimates of the births between each sampling occasion are skewed (Buckland and Garthwaite 1991). This is as a result of the Central Limit Theorem that applies when the individual estimates of recruitment are added.

In the bootstrap approach, the observed experimental data are either simulated (parametric bootstrap) or resampled (nonparametric bootstrap) to create a series of "pseudo-experiments" (Buckland 1980; Buckland and Garthwaite 1991). Each of these "pseudo-experiments" is analyzed to obtain an estimate. The resulting distribution of the estimates may provide good information on the bias and precision of the estimator. Sykes and Botsford (1986) used a parametric bootstrap to obtain an estimated sampling distribution of the escapement and

TABLE 4. Parameter values used in the baseline simulation and expected statistics.

Sample occasion	New entrants (B_i^*)	Survival rate (ϕ_i)	Capture rate (p_i)	Average sample size $E(n_i)$	Average marked of n_i $E(m_i)$	Average recoveries $E(r_i)$
0	100					
1	150	0.60	0.25	25.0	0.0	6.8
2	250	0.60	0.25	44.4	3.8	12.1
3	200	0.60	0.25	75.6	8.3	20.5
4	0	0.60	0.25	84.5	15.1	22.9
5	0	0.60	0.25	50.7	19.5	13.6
6	350	0.60	0.25	30.4	16.4	8.0
7	100	0.60	0.25	86.9	11.9	21.5
8	50	0.60	0.25	71.6	18.4	15.6
9	0	0.60	0.25	52.8	19.0	7.9
10		0.60	0.25	31.7	16.5	
Total	1200					

TABLE 5. Summary of the point estimates from the baseline simulation. Column numbers (at top) are cited in the text (ne = nonestimable).

1	2	3	4	5	6	7
		Closed-form MLEs		Less-biased closed-form estimators		
Sample occasion	B_i^*	Uncensored	Censored	Uncensored	Censored	Constrained MLE
0+1	250	282	282	209	209	260
2	250	260	271	252	254	250
3	200	200	212	205	209	169
4	0	−4	33	3	32	22
5	0	−5	14	−4	12	12
6	350	387	387	343	343	363
7	100	104	115	108	114	104
8	50	41	52	41	48	40
9	0	ne	ne	ne	ne	ne
Total	1200	1266	1366	1157	1219	1221

formed confidence intervals based upon percentiles of this bootstrap sampling distribution as suggested by Buckland (1980). A problem with the parametric bootstrap approach occurs when some estimates are invalid, e.g., how is a population simulated that has a survival rate greater than 1 or negative births? Simply setting the out-of-range estimates to the boundary points will result in bias as previously shown. Buckland and Garthwaite (1991) demonstrated that a nonparametric bootstrap method, conditional upon the number of animals actually caught, may be used to find confidence intervals with good coverage. This method has the advantage that it is not necessary to simulate the birth and death processes between sampling occasions, nor is it necessary to simulate the animals never caught, which is necessary in a parametric bootstrap approach. The major impediment to using bootstrap procedures is the amount of computing required.

4. Simulation Experiment

Two sets of simulation experiments were performed. The first set compared the properties of five estimators of escapement under ideal conditions; the second set compared the constrained MLE in situations where the underlying assumptions were violated.

4.1. Comparing Estimators when the Assumptions are Satisfied

In the first set of simulations, recruitment to the population and the mortality rate were assumed to be uniform between sampling occasions. No losses on capture were modeled. The number of new entrants to the population, the capture rates, and the survival rates were chosen based upon the results from the 1989 data analyses. These and the expected number of marked animals and subsequent recoveries are presented in Table 4.

Parameters were estimated five different ways (Table 5) in each of the 200 simulations: the closed-form MLEs (Table 2), with and without censoring of negative estimates of births; the less-biased closed-form estimators (Table 2), with and without censoring of negative estimates of birth; and the MLEs when the estimates are constrained to lie in the parameter space (Appendix C). For each method, the escapement was estimated using equation (2). The average values of the point estimates of recruitment between sample occasions and the estimates of the escapement over the simulations are shown in Table 5.

As expected, simply censoring negative estimates increased the average value of the estimator (column 4 versus column 3; column 6 versus column 5), particularly if the number of births was close to zero (sample occasions 4 and 5). Censoring increased the estimates of escapement by about 100 fish for the

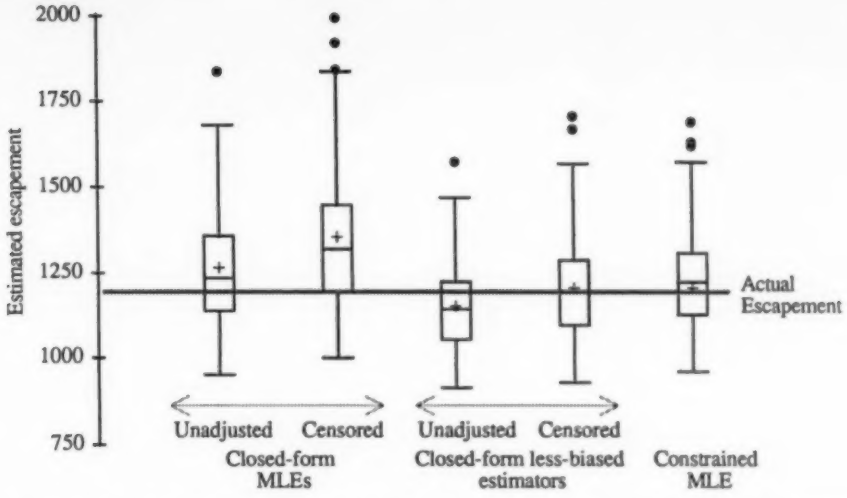


FIG. 1. Box plots of the distribution of the estimated escapement from the baseline simulation using the five methods described in the text.

closed-form MLE and by about 60 fish for the less-biased estimator. The uncensored closed-form MLEs are positively biased (column 3 versus column 2). The uncensored less-biased estimators are nearly unbiased (column 5 versus column 2) at all sample occasions except, as noted following equation (2), the initial population size (B_0^*) was underestimated. This accounted for the underestimation of the escapement.

The constrained MLEs (column 7) exhibit features from columns 3 to 6. Because the estimator constrains all estimates of births to be positive, the estimates at sampling occasions with small numbers of births are positively biased. However, the numerical optimization then forces estimates of births at neighboring occasions (sampling occasions 3 and 6) to be reduced compared with the unconstrained MLEs. Consequently, the overall estimate of escapement lies between that of the closed-form MLE and that of the less-biased estimator.

Box plots of the escapement estimates (Fig. 1) indicate that the closed-form MLEs are more variable than the other estimators. The plots show the positive bias introduced by censoring and that the distributions of the less-biased estimators and the constrained MLE are nearly symmetric. The distribution of the constrained MLE appears to be intermediate between the closed-form MLE and the less-biased estimator.

SEs were computed for the estimates of the recruitment between sample occasions and for the escapement (Table 6) by using the closed-form asymptotic expressions of Appendix C, and using the true parameter values (these of course are never known in practice); finding the standard deviation of the estimates among the simulations (this is an estimate of the correct SE for an estimator, since, by definition, it measures the changes in the estimates over repeated experiments with the same parameter values); using the closed form expressions of Appendix C, and using estimates in place of the true value of the parameters as is usually done in practice (this was done using the closed form MLEs, the closed-form less-biased estimators, and the constrained MLEs; in the latter, the parameter estimates were used to estimate the $E[m_i]$ and other expected sample statistics that appear in the asymptotic formulae); and using the inverse of the information matrix found at the final step of the numerical optimization of the likelihood. These were then averaged over all the simulations.

The closed-form MLEs are more variable than the asymptotic results predicted early in the experiment when there are few marked animals in the population (column 3 versus column 2). This leads to an over dispersion of the escapement estimates as seen in Fig. 1. The estimated SEs appear to reflect this increased variability (column 4 versus column 3). The actual variation of less-biased estimators matches the asymptotic results well (column 5 versus column 2), and the estimated SEs appear to be consistent (column 6 versus column 5). The constrained MLE also performs well (column 7 versus column 2; columns 8 and 9 versus column 7). For all methods, it is important that the estimated SE be a good estimate of the actual variation in the estimator (column 4 versus column 3; column 6 versus column 5; columns 8 and 9 versus column 7) rather than matching the asymptotic results (column 2). Consequently, Table 7 indicates that the estimated SE are reliable for the less-biased estimators and the constrained MLEs.

A normal probability plot of the constrained MLEs of escapement (Fig. 2) shows that the estimator is approximately normally distributed between normal scores of -2 and $+2$ as a consequence of the Central Limit Theorem. This implies that confidence intervals should perform well. The coverage of a nominal 95% confidence interval for the escapement was also estimated (Table 6). Because the closed-form MLEs and the less-biased estimators were positively and negatively biased, respectively, the coverage failed to achieve the nominal level despite the estimated SE being a good estimate of the actual variability. The constrained MLE has an estimated coverage close to the nominal level.

The simulations confirmed the properties of our proposed estimator as outlined in Section 3. In particular, the closed-form MLEs are positively biased; the unconstrained less-biased estimators perform well except as noted at the start of the experiment; the constrained MLE is intermediate between the closed form MLE or the less-biased estimator; censoring negative estimates of birth leads to a positive bias; the closed-form MLEs are more variable than expected at the beginning of the sam-

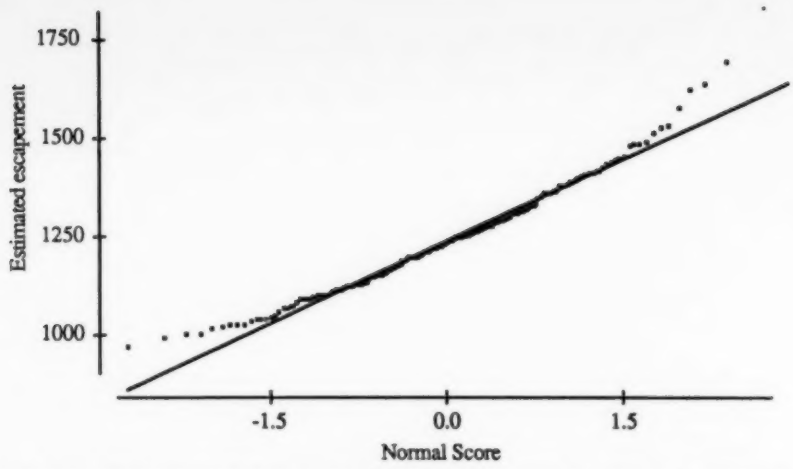


FIG. 2. Normal probability plot of constrained MLE of the escapement. The straight line represents the expected values of the estimated escapements if the sampling distribution was actually normally distributed. Distributions of the uncensored estimates were similar and are not shown here.

TABLE 6. Comparison of the SEs of the estimates and of the coverage of a 95% confidence interval from the baseline simulation. Column numbers (at top) are cited in the text.

1	2	3	4	5	6	7	8	9
		Uncensored closed-form MLEs		Uncensored less-biased closed-form estimators		Constrained MLE		
Sample occasion	Asymptotic SE from formula	Actual SD from simulations	Average estimated SE from formula	Actual SD from simulations	Average estimated SE from formula	Actual SD from simulations	Average estimated SE from inv. of info. matrix	Average estimated SE from formula
0+1	118	176	177	91	119	134	135	139
2	125	263	217	154	160	155	148	136
3	120	140	156	129	134	92	100	95
4	74	54	90	82	80	37	33	55
5	36	25	44	36	38	23	19	62
6	123	150	147	117	129	122	124	130
7	100	94	120	97	107	89	85	92
8	63	65	73	61	64	43	45	37
Escapement	125	197	170	132	153	139	142	147
coverage			0.92		0.91		0.96	

pling experiment; the closed-form, large-sample estimates of the variance work reasonably well; and the estimates of the SE obtained by inverting the information matrix give good estimates of precision except when the number of births is small, when they underestimate the true SE.

4.2. Effects of Violations of the Assumptions

The preceding section indicated that the constrained MLE performed well when the assumptions of the model were satisfied. However, assumptions are never completely satisfied in real experiments. In this section, we investigate, through simulation, the likely effects of violations of the key assumptions of the Jolly–Seber model, i.e., the assumptions of homogeneity in the survival and capture rates among fish.

Five simulations were conducted as described in Table 7. The extent and types of violations were chosen to be realistic biologically. Two hundred replications of each simulation were generated and the constrained MLEs were computed for each replicate of each simulation. Box plots of the estimated escapements are given in Fig. 3.

Pollock et al. (1990, section 4.6) indicated that pure heterogeneity in survival rates that is independent of capture rates (simulation 2) leads to a positive bias in survival rate and population size estimates. Since net births are essentially estimated as $\hat{B}_i = \hat{N}_{i+1} - \hat{\phi}_i\hat{N}_i$, a positive bias in $\hat{\phi}_i$ will lead to a negative bias in $\hat{B}_i$. The positive bias in $\hat{\phi}_i$ will also lead to a negative bias in the adjustment factor (Table 3), which implies that estimates of gross births will be negatively biased. This is confirmed by comparing the results of simulation 2 to the baseline simulation (Fig. 3); estimates of escapement have been reduced. The amount of bias caused by this degree of heterogeneity is only about 0.50 of a SE of the baseline estimates.

When "newer" fish have higher survival rates than "older" fish (simulation 3), survival rates will be biased downwards. Survival rates are essentially estimated by the ratio of recoveries from previously marked ("older") fish to those of newly

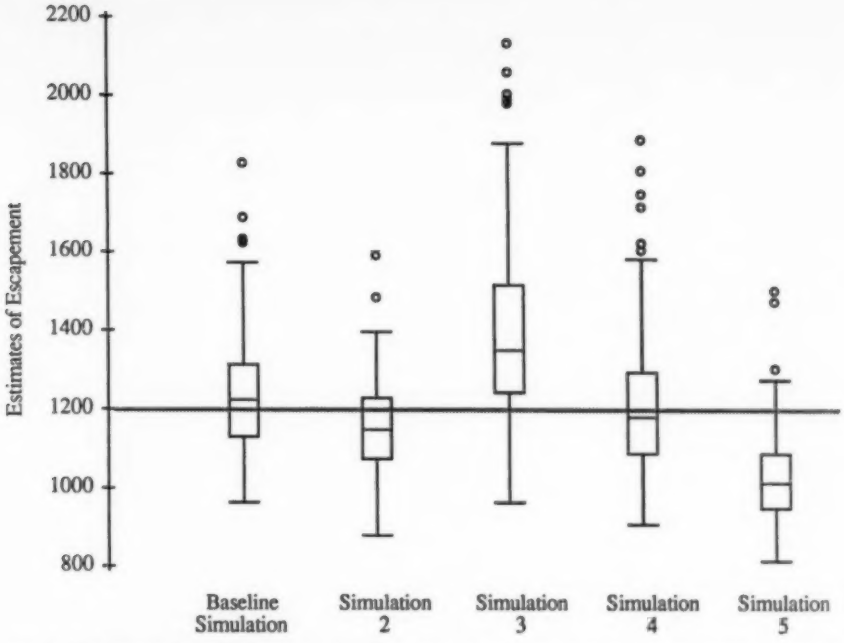


FIG. 3. Box plots of the estimates of escapement from the five simulations (see text for description of simulations). The horizontal line is the actual escapement.

TABLE 7. Description of the five simulations used to investigate the effects of violations of the assumptions.

Simulation	Description
Baseline	As performed in Section 4.1 using the parameter values given in Table 4.
2	Pure heterogeneity in survival rates among fish. As each fish enters the experiment, its survival rate was selected from a uniform distribution on the interval [0.40, 0.80]·wk^{-1} and remained at this level until death. Capture rates were homogeneous for all fish as given in Table 4
3	Heterogeneity in survival rates among fish caused by increasing mortality after entering the stream. When fish enter the experiment, they have initial survival rates of 0.60·wk^{-1}. This declines by 20%·wk^{-1}, e.g., fish that entered in week 1 would have a survival rate of 0.48 in week 2; fish that first entered in week 2 would have a survival rate of 0.60
4	Declining survival rate after entry with increased recapture rates for "older" fish. Survival rates decline as in (3), but recapture rates increase as survival decreases, since "weaker" fish are more easily recaptured. The product of survival and recapture was set to 0.25, e.g., a fish with a survival rate of 0.48 would have a recapture rate of 0.52
5	Pure heterogeneity in recapture rates. As in the baseline simulation but each fish is assigned a recapture rate from a uniform distribution on the interval [0.05, 0.45], which remain fixed for the entire experiment

marked fish. The recoveries of the previously marked fish will be reduced because their survival rates are lower leading to the negative bias. Consequently, the adjustment factors will be biased upwards (Table 3) leading to a positive bias relative to the baseline simulation (Fig. 3). The size of the bias for this simulation was on the order of 1 SE.

When "older" fish are also more prone to recapture (simulation 4), two opposing effects are active. Heterogeneity in survival rates related to the "age" of the fish causes a positive bias; heterogeneity in capture rates causes a negative bias (Pollock et al. 1990, section 4.6). In this simulation, the two forces fortuitously nearly cancel leading to almost a zero bias (Fig. 3).

Lastly, pure heterogeneity in capture rates (simulation 5) leads to negative bias. In practice, the bias is small when the average capture rate is high, say >0.50 (Pollock et al. 1990, section 4.6). As well, the practice of pooling several closely spaced sampling occasions into one reduces the heterogeneity because the multiple captures of the more catchable fish are treated as a single capture. In this simulation, the heterogeneity is large relative to the average capture rate (0.25) which leads to a large negative bias (Fig. 3).

These simulations suggest that our proposed estimator is robust to moderate violations of assumptions. They also demonstrate how simulations may be used to assess the consequences of likely violations in real experiments.

5. Application of the Method to Estimate Coho Salmon Escapements to the Chase River in 1989 and 1990

5.1. Methods and Suitability of the Jolly–Seber Model

A capture–recapture study to estimate coho salmon escapements to the Chase River, British Columbia, was performed in 1989 and 1990. The Chase River is a small stream, flowing northeast out of the Colliery Park Dams in south Nanaimo for approximately 4.5 km, entering the Strait of Georgia in the Nanaimo River estuary. Discharge ranges from

less than 0.2 $m^3 \cdot s^{-1}$ at summer low flows to greater than 30 $m^3 \cdot s^{-1}$ during winter floods.

During each week of the fall salmon run, water conditions permitting, the entire river was sampled, usually over 2 d, and the entire week's sampling effort was considered to be a single pass. Early in the run, prior to the salmon dispersing to the spawning areas, large pools in the lower 2 km of river were tangle-netted weekly. Tangle-netting was performed using 25 m × 3 m × 3 cm mesh gill nets. These nets, when operated as seines, tangled both adult and jack (precocious male) coho, although fish did not become trapped by the opercula as would happen in conventional gillnetting. After the first fall freshet event, flows were often too high for netting to be practical, the fish moved into shallower areas of the river, and consequently, electrofishing was used to capture most fish. Electrofishing was performed by crews of three, two people carrying large-frame (1.25 m hoop), fine-meshed (1 cm) landing nets and the third person operating the electrofisher. Electrofishing was carried out using Smith Root Mk. 11A and 12A shockers, operating at 200–300 V pulsed DC at 30 Hz. Near the end of each field season, when no live fish were observed, several walks of the entire stream were conducted over a 3-wk period to recover carcasses (dead pitch).

Individually numbered Ketchum No. 1 operculum tags (Ketchum Manufacturing Sales Ltd., 396 Berkley Ave., Ottawa, Ont., Canada K2A 2G6 were applied to the operculum of each live fish captured, and the sex, degree of sexual maturity, date, and location of capture were recorded. The tag number of each recaptured fish was recorded. Multiple recaptures within a week were treated as a single capture, and carcass recoveries were recorded as losses-on-capture. Once counted, carcasses were cut in half to prevent recounting. Data from the dead-pitch stream walks at the end of the experiment were pooled to form one final sampling "week."

In this study, sampling was not instantaneous, but took place over 2–3 d for most of the study and over 3 wk for the final dead pitch. The former instance is small enough that the violation of the assumption of instantaneous sampling is not judged to be serious. Since the latter occurs at the end of the experiment, and involves carcass recoveries only, its extended period has little effect on the estimates.

Catchability was presumably unequal among fish. This was due in part to the location of the fish, the type of capture method, variability in size and maturity of the fish, and any previous encounters with a capture method. The pools in the lower river were more intensively sampled by net than those in the upper river early in the study. During electrofishing, those few fish that were under cover in runs but not in deep pools were very susceptible to capture. Fish in shallow runs during and after spawning were more visible and less timid, and again were easier to catch by electrofishing. The skin of silver-bright fish is more conductive than that of darker, more mature fish, which makes them easier to catch using electrofishing. However, any multiple captures within a week were recorded as a single capture within the week. Consequently, the bias caused by heterogeneity among animals within any week are expected to be slight (refer to Section 4.2).

Little tagging mortality occurred. Natural survival rates likely differed among animals within any week because the animals were at differing stages of maturity. Survival rates were likely highly correlated with maturity; bright, early fish have been recorded alive some 50 d after first capture, but fish that were actively spawning were commonly found as carcasses within 1 wk (R.E. Bailey, unpublished data). As shown in Section 4.2, pure heterogeneity in survival rates will have a negative bias on the estimates of escapement. This type of heterogeneity occurs if a mixture of fish at different stages of maturity enter each week. If, however, fish enter the stream at a relatively fixed maturity level, this corresponds to heterogeneity as explored in simulation 3 of Section 4.2, and the resultant estimate of escapement will have a positive bias. Heterogeneity in capture rates will lead to a negative bias. In practice, a mixture of different types of heterogeneity occur, some with negative and some with positive biases.

Based upon 3 yr of experimental data, tag loss was less than 1%. It is not likely that tags were overlooked, since all crews were experienced.

The use of carcass recoveries and normal recoveries will not lead to serious bias if the number of carcass recoveries is small, or if carcasses do not remain in the stream long after death. If carcasses can remain in the stream for long periods after death, estimates of survival will be inflated because carcass recoveries will be treated as if the fish had survived to the time of carcass recovery. In this experiment, the number of carcass recoveries was small at all sampling occasions except the last. The last 3 wk of dead pitch when no live fish were observed were pooled into one last sampling occasion where parameters of survival and capture are confounded, not estimated in any case, and the number of entries was assumed to be zero.

We assumed that recruitment between sampling occasions occurred uniformly. Since the weekly survival rates were over 50%, the choice of distribution for the recruitment was not critical (Table 3) and we used equation (2) to estimate the escapement.

5.2. 1989 Escapement Estimates

The run to the Chase River in 1989 consisted of two pulses of fish. The first pulse began to arrive around the last week of September (week 1) and peaked with the first heavy rains in mid-October. A second smaller pulse arrived in mid-November.

The summary statistics for this experiment (in Jolly–Seber notation) are as follows. Only carcasses were recovered in weeks 10, 11, and 12; data for these have been grouped together.

Week	Number of captures n_i	Number of marks m_i	Losses on capture	Released after marking R_i	Subsequently recaptured r_i	Seen before i, not at i, and after i z_i
1	5	0	0	5	1	0
2	80	0	0	80	27	1
3	35	12	1	34	19	16
4	97	14	25	72	31	21
5	84	25	6	78	34	27
6	67	39	11	56	14	22
7	51	28	14	37	5	8
8	9	6	9	0	0	7
9	30	0	1	29	7	7
10+	18	14	18	0	0	0

Goodness-of-fit tests (Pollock et al. 1990) failed to detect any serious evidence of failure of the assumptions of equal catchability or survival, except for some evidence of differential mortality between previously marked and newly marked fish. Previously marked animals may have entered the river earlier, and hence were more likely to be spawning or have finished spawning.

More serious problems exist, however. In weeks 2 and 9, no previously marked fish were captured. This implies that the MLEs of abundance (and consequently of births) are infinite.

TABLE 8. Estimates and estimated SEs computed using three methods for the 1989 Chase River study (ne = nonestimable).

Sample occasion	Unconstrained MLE		Less biased estimators		Constrained MLE	
	Estimate	Estimated SE	Estimate	Estimated SE	Estimate	Estimated SE[a]
			Recapture rates (p_i)			
1 & 2	ne		ne		ne	
3	0.30	0.086	0.30	0.086	0.30	0.086
4	0.22	0.064	0.23	0.065	0.25	0.037
5	0.29	0.063	0.29	0.063	0.33	0.050
6	0.31	0.075	0.32	0.077	0.29	0.071
7	0.32	0.126	0.36	0.132	0.32	0.126
8 & 9	0.17	0.092	0.19	0.098	0.46	0.118
10+	ne		ne		ne	
			Survival rates (ϕ_i)			
1 & 2	0.48	0.093	0.47	0.092	0.48	0.093
3	1.00	0.195	1.00	0.195	0.98	0.181
4	0.72	0.124	0.72	0.123	0.69	0.114
5	0.91	0.203	0.88	0.197	0.93	0.211
6	0.61	0.247	0.56	0.227	0.61	0.247
7	0.36	0.190	0.37	0.191	0.23	0.106
8 & 9						
			Population size (N_i) with SE conditional upon N_i not N_i			
1 & 2	ne		ne		ne	
3	118.5	31.6	110.8	29.0	118.5	29.9
4	434.9	119.8	404.5	110.1	392.3	47.4
5	292.1	59.3	281.0	56.4	254.1	30.7
6	218.2	50.1	208.4	47.5	231.5	51.8
7	158.8	60.6	141.1	52.5	158.8	59.5
8 & 9	227.5	117.9	184.3	93.7	84.6	19.2
10+	ne		ne		ne	
			Net births (B_i) with SE conditional upon N_i not B_i			
1 & 2	ne		ne		ne	
3	317.2	121.3	294.9	102.8	277.3	43.7
4	−3.0	88.0	9.0	78.6	0.0	0.0
5	−41.5	45.7	−34.2	41.5	0.0	0.0
6	33.4	26.1	29.8	21.8	25.3	23.1
7	174.8	108.9	137.5	81.9	51.5	13.9
8 & 9	ne		ne		ne	
			Gross births (B_i^*)			
0 & 1 & 2	167.6	43.7	157.7	40.5	167.6	41.6
3	316.8	102.4	295.1	94.3	280.4	43.0
4	−3.5	100.0	10.6	92.2	0.0	0.0
5	−43.5	46.9	−36.4	44.2	0.0	0.0
6	42.4	29.1	39.2	26.3	32.2	28.0
7	277.8	145.9	217.6	114.8	98.5	29.4
8 & 9	ne		ne		ne	
Escapement	757.6	162.4	683.7	131.4	578.7	49.7 64.7[b]

[a]When estimates of the parameters are constrained to lie on the bounds of the parameter space, the estimated SE is reported as 0.00
[b]This estimate of the SE is obtained by evaluating the asymptotic formula using the constrained MLE.

The bias-adjusted estimates are not much better. Consequently, sample occasions 1 and 2 and 8 and 9 were pooled. Animals seen at least once in a pooled period were recorded as being seen once. This led to the revised summary statistics:

Week	Number of captures n_i	Number of marks m_i	Losses on capture	Released after marking R_i	Subsequently recaptured r_i	Seen before i, not at i, and after i z_i
1 and 2	85	0	0	85	28	0
3	35	12	1	34	19	16
4	97	14	25	72	31	21
5	84	25	6	78	34	27
6	67	39	11	56	14	22
7	51	28	14	37	5	8
8 and 9	39	6	10	29	7	7
10+	18	14	18	0	0	0

These summary statistics were used to compute the unconstrained MLEs, the unconstrained less-biased estimates, and the constrained MLEs. The estimates (Table 8) confirmed the two pulses of fish, one early and the second late in the season. The fish had a survival rate over 70%·wk^{-1}. Note that the pooled estimates for weeks 1 and 2, and 8 and 9 refer to the 2-wk periods. The high survival rate implies that estimates of total recruitment between sampling occasions are not very sensitive to the assumed distribution of recruitment within the interval (Table 3). The estimated recapture rate (Table 8) appears to have been roughly constant at about 30%·wk^{-1} except at the end of the season when mainly carcasses were recovered. The three estimates of the escapement have a wide range, the unconstrained MLE of 758 being the highest and the constrained MLE of 579 being the lowest. These differences are largely artifacts of this particular data set caused by differences in confounded parameters at the end of the experiment. For example, using the estimators from Table 2, we find that

$$\phi\widetilde{\widetilde{8\&9}}p_{10} = r_{8\&9}/R_{8\&9} = 7/29 = 0.24$$

$$B\widetilde{\widetilde{8\&9}}/\phi_{8\&9} = \frac{n_{10}}{\phi_{8\&9}p_{10}} - (\tilde{N}_{8\&9} - n_{8\&9} + R_{8\&9})$$

$$= \frac{18}{0.24} - (184.3 - 39 + 29) = -99.3.$$

This estimate is out-of-range. If this out-of-range estimate is added to the escapement estimate, the result is now much closer to the estimate using the constrained MLEs. The contrained MLE ensures that all estimates are within the range of the parameter space, i.e., constrains the last term to be nonnegative. The high negative sampling correlation between neighboring estimates of births implies that raising the estimate of the last "confounded parameter" to zero must decrease neighboring estimates. Consequently, we believe that the constrained MLE is a more realistic estimate of the escapement. If no correction for mortality of entering fish is made, the estimate of escapement found by adding estimates of $N_2, B_2, \ldots$, and B_{s-2} is about 470 fish, a presumed undercount of almost 20%.

The estimated SEs of the unconstrained MLE and unconstrained less-biased estimator are similar and both are much larger than the estimated SE for the constrained MLE. A large part of the difference is again an artifact of the data and is caused by the out-of-range confounded parameter estimate. A small part of the difference is due to the numerical method used to constrain parameter estimates which reports the estimated SEs of those constrained estimates as zero. This is seen when the SE of the constrained MLE is evaluated using the asymptotic formulae; this leads to a slight increase in the estimated SE. A 95% confidence interval for the escapement is estimated to be 578.7 ± 1.96 (64.7) or from 452 to 705 fish.

The constrained MLE was also computed ignoring carcass recoveries. We obtained an estimated escapement of 638 with an estimated SE of 70. A nonparametric bootstrap of the constrained MLE gave an estimated mean escapement of 630 with an estimated SE of 47. The estimated 95% confidence interval was found to be from 559 to 727. The nonsymmetric bootstrap confidence interval would seem to indicate that the distribution of the constrained MLE is skewed, a likely result of the problems identified in the data. Both estimates are similar to our result.

Finally, a pooled Petersen estimate was computed by treating the initial tagging of a fish over all sampling occasions as the "first" sample and carcass recoveries over all weeks as the "second" sample. We obtained an estimated escapement of 524 with an estimated SE of 43. However, as discussed by Seber (1982, p. 437), this "pooled" estimate will be consistent if a constant proportion of the carcasses is sampled and/or if a constant proportion of the population is marked. After the fact, this may have occurred because the estimates of p_i (Table 8) are very similar.

These data had numerous problems that made their analysis difficult. First, there were no marks recovered in two of the sample periods which led to infinite estimates of escapement. This problem was resolved by pooling adjacent sample periods. The recruitment in the last sample period was still not estimable, but the negative estimate of the confounded parameter had a serious effect on the closed-form MLE and less-biased estimator. For this data set, the constrained MLE was the most appropriate estimate. Similar effects were seen on the estimated precisions. Again, the asymptotic formula evaluated at the constrained MLEs was the most appropriate. Despite these problems, the estimate of escapement was quite precise with a CV of about 11%.

5.3. 1990 Escapement Estimates

The approach was repeated in the fall of 1990 and gave data with fewer problems than the 1989 experiment. This example will illustrate that under these conditions, all methods will give similar results.

The returning run of coho salmon in 1990 consisted of three pulses of fish. The first group arrived in late September (week 1), and numbers of fish entering the river peaked by mid-October. A second pulse arrived in early November, and a smaller, late pulse arrived at the end of the first week of December. The field data gave rise to the following summary statistics:

Week	Number of captures n_i	Number of marks m_i	Losses on capture	Released after marking R_i	Subsequently recaptured r_i	Seen before i, not at i, and after i z_i
1	3	0	0	3	1	0
2	10	1	0	10	4	0
3	16	3	0	16	5	1
4	124	3	3	121	36	3
5	82	30	3	79	13	9
6	125	12	20	105	22	10
7	117	18	36	81	16	14
8	51	16	12	39	7	14
9	63	18	31	32	1	3
10+	14	4	14	0	0	0

Goodness of fit tests (Pollock et al. 1990) failed to detect

TABLE 9. Population estimates and estimated SEs computed using three methods for 1990 Chase River study (ne = nonestimable).

Sample occasion	Unconstrained MLE		Less biased estimators		Constrained MLE	
	Estimate	Estimated SE	Estimate	Estimated SE	Estimate	Estimated SE[a]
			Recapture rates (p_i)			
1	ne		ne		ne	
2	1.00	0.000	1.00	0.000	1.000	0.001
3	0.48	0.303	0.51	0.303	0.48	0.303
4	0.23	0.146	0.23	0.148	0.34	0.052
5	0.35	0.105	0.37	0.106	0.35	0.103
6	0.20	0.075	0.21	0.077	0.20	0.075
7	0.20	0.068	0.21	0.070	0.22	0.053
8	0.17	0.071	0.19	0.076	0.16	0.066
9	0.16	0.155	0.27	0.228	0.16	0.155
10+	ne		ne		ne	
			Survival rates (ϕ_i)			
1	0.33	0.272	0.33	0.272	0.33	0.272
2	0.62	0.329	0.58	0.313	0.62	0.329
3	0.68	0.303	0.68	0.304	0.58	0.225
4	0.65	0.172	0.62	0.166	0.65	0.175
5	0.48	0.147	0.44	0.147	0.45	0.147
6	0.58	0.169	0.57	0.165	0.56	0.151
7	0.62	0.239	0.58	0.223	0.63	0.242
8	0.97	0.972	0.62	0.600	0.97	0.971
9	ne		ne		ne	
			Population size (N_i) with SE conditional upon N, not N_i			
1	ne		ne		ne	
2	10.0	3.2	5.5	est < 0	10.0	0.0
3	33.1	20.6	24.8	13.3	33.1	19.8
4	540.8	343.1	402.9	242.5	363.6	48.5
5	231.5	66.6	218.0	62.1	235.6	66.9
6	622.2	228.3	563.0	204.5	622.2	227.4
7	577.7	188.9	531.2	172.5	532.2	120.6
8	299.6	119.6	263.1	104.2	312.9	119.8
9	399.0	389.2	227.4	213.0	398.9	388.7
10+	ne		ne		ne	
			Net births (B_i) with SE conditional on N, not B_i			
1	ne		ne		ne	
2	26.9	21.3	21.6	13.0	26.9	17.6
3	518.2	368.2	385.9	243.4	344.4	47.6
4	−116.0	218.3	−30.7	150.1	0.0	0.0
5	520.1	221.6	467.2	187.6	518.2	208.1
6	227.3	176.0	223.8	151.9	194.8	128.6
7	−35.6	103.3	−23.7	86.4	0.0	0.0
8	118.8	150.5	71.9	84.4	105.8	135.6
9	ne		ne		ne	
			Gross births (B_i^*)			
+1	16.5	7.5	9.1	est < 0	16.5	3.7
2	33.8	19.3	27.9	15.5	33.8	19.3
3	624.0	356.0	463.6	258.6	446.0	89.7
4	−143.1	271.9	−38.6	189.6	0.0	0.0
5	757.4	259.4	681.4	233.3	754.8	258.3
6	294.3	201.8	293.5	183.3	256.6	164.8
7	−44.9	128.2	−30.7	112.6	0.0	0.0
8	120.3	113.4	90.5	80.8	107.2	102.8
9	ne		ne		ne	
Escapement	1658.3	273.0	1496.7	236.7	1614.8	193.0 238.1[b]

[a]When estimates of the parameters are constrained to lie on the bounds of the parameters space, the estimated SE is reported as 0.00
[b]This estimate of the SE is obtained by evaluating the asymptotic formula using the constrained MLE.

any violation of assumptions. The numbers of recoveries were marginally adequate at the start and end of the experiment, but no pooling was done, since the number of births at the start and end was small and any bias resulting from the small counts was felt to be acceptable.

The resulting estimates (Table 9) confirm three pulses of recruitment. Survival rates in all weeks except the first were approximately 60%·wk^{-1}, which implies that the choice for the assumed distribution of recruitment between sampling occasions was relatively unimportant (Table 3). The small number of new fish in week 1 implies that any biases from using an incorrect recruitment distribution will be small. Note that the high survival rate in week 8 resulted from treating carcass recoveries in week 9 as losses on capture. Recapture rates appeared to be roughly constant over time. Escapement estimates are more similar than those calculated from the 1989 results, since there were fewer problems with out-or-range estimates of either the identifiable or confounded parameters. The 1990 estimated escapement ranged from 1497 from the less-biased estimator to 1658 for the unconstrained MLE. The estimate of the SE of the constrained MLE is slightly smaller than the estimated SEs of the other methods due to the constraints of the recapture rate at time 2, and the constraints to keep the estimated number of births admissible. For these data, either the unconstrained less-biased estimate or the constrained MLE could be used as a good estimate of the escapement, and it is felt that the estimates of SE are reliable. Using the constrained MLEs, a 95% confidence interval for the escapement is found to be 1615 ± 1.96 (238.1) or between 1148 and 2081 fish. In 1990, more fish were marked than in 1989, but fewer recaptures occurred giving less precise estimates than the previous year (CV of about 15%).

The constrained MLE of escapement, ignoring carcass recoveries, was 1611 with an estimated SE of 312, very similar to the above results. The increase in the estimated SE is expected because information is lost by ignoring carcass recoveries. A nonparametric bootstrap applied to the constrained MLE gave an estimate for the escapement of 1650 fish with an estimated SE of 188 fish and a 95% confidence interval of 1359–2132 fish. The results are consistent with the initial analysis. Finally, a Petersen estimate computed as for the 1989 data gave an estimated escapement of 1738 fish with an estimated SE of 260 fish. Given the imprecise estimates of the capture rates (Table 9), it again may be possible that initial capture rates are constant over time.

6. Summary

A reluctance by fishery workers to use open-population capture–recapture methods to estimate escapement is due, in part, to uncertainty of how to account for fish that enter and die before a sampling occasion and how to estimate recruitment at the start and end of the experiment. In this paper, we demonstrated that simple modifications to the Jolly–Seber estimates can yield useful estimates of escapement (equation (2)). These modifications depend upon the assumed distribution of recruitment between sampling occasions. If survival between sampling occasions is low, the adjustment factors vary considerably; if the survival rates are high (≥50%), then the choice of recruitment distribution is not critical. This implies that an experiment should be designed to ensure high survival between sampling occasions. The study should also be planned so that few entries occur outside their temporal bounds.

As well, uncertainty existed about a number of technical problems that often occur in a particular dataset (e.g., the 1989 Chase data). The closed-form MLEs are biased and the bias is greatest when the sample sizes are small. The less-biased estimators are an improvement but only work well when m_i and r_i are at least 10. Consequently, when data are sparse, we recommend the pooling of adjacent sample occasions. The practice of simply censoring out-of-range estimates is to be avoided because this leads to a positive bias in the estimates of recruitment and of the escapement. Either negative estimates of births should be used as given or numerical methods should be used to maximize the likelihood subject to the boundary constraints (Appendix C). The latter is particularly important if estimates of the confounded parameters at the end of the experiment are out of range. The large-sample closed-form estimates of SE provide good estimates of the precision of the escapement when the sample sizes are reasonably large. They are suspect when some estimates are out of range regardless of sample size. Numerical optimization methods typically report SEs of constrained parameters as zero which leads to under-estimates of the SEs when the inverse of the information matrix is used to estimate precision. We recommend that the asymptotic formulae be evaluated at the constrained MLE. Finally, the estimates of the escapement are approximately normally distributed due to an application of the Central Limit Theorem and the large sample confidence intervals are expected to perform well.

It is important that the usual assumptions for capture–recapture studies be valid for a particular experiment, in particular, the assumptions of homogeneity of survival and capture rates. Good discussions of the effects of violations of the assumptions of capture–recapture experiments and of planning capture–recapture experiments can be found in Sykes and Botsford (1986) and Pollock et al. (1990, sections 4.6 and 8.7).

The precision of the escapement estimates depends upon several aspects of the study. In general, precision will increase as the number of marked animals recovered increases. Higher recoveries of marked animals may be obtained by increasing the capture rates at each sampling occasion (not necessarily possible) or by increasing the number of sampling occasions relative to the age of the animal (e.g., more frequent sampling). Because of the complexity of the estimation process, it is not possible to give rules of thumb for sample sizes. We recommend that the experimenter use simulations to generate sets of "dummy data" and then analyze these "data" to determine if precision is adequate. This method may also be used to investigate the effects of varying effort and other aspects of the study upon precision.

7. Software

Computer software to find the constrained MLE is available from the first author. It is written in FORTRAN 77 and requires subroutines to invert matrices commonly found in numerical libraries. It is also integrated into POPAN (Arnason and Baniuk 1978).

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Appendix A. Obtaining Adjustment Factors

Let B_i^* be the total recruitment and ϕ_i be the survival rate between two sampling periods. Without loss of generality, we may take the time interval between the two sampling points as being [0,1].

A uniform mortality rate in the interval implies that the probability that an animal that enters the population at a time t will survive to the next sampling period at time 1 is $\phi^{(1-t)}$.

Suppose that recruitment follows a probability distribution $f(t)$. Then the net recruitment can be computed as

$$B_i = \int_0^1 B_i^* f(t)\, \phi^{(1-t)}\, dt.$$

This equation is then inverted to solve for B_i^* in terms of B_i.

For a uniform recruitment, $f(t) = 1$. For the left triangular distribution, $f(t) = 2(1 - t)$. For the right triangular distribution, $f(t) = 2t$.

Appendix B. Approximating the Bias Caused by Censoring Negative Estimates

Suppose that an estimator $\hat{\theta}$ has an approximate normal distribution with a mean of θ and a standard deviation of σ and let $\hat{\theta}' = \max(\hat{\theta},0)$ be the censored estimator where estimates below 0 are replaced by 0. Then the expected value $E[\hat{\theta}'] = \sigma/\sqrt{2\pi}\, \exp(-0.5(\theta/\sigma)^2) + \theta(1-\Phi-\theta/\sigma))$ where $\Phi(\cdot)$ is the standard normal cumulative distribution. The extent of the potential bias is shown in the following table:

θ/σ	$E[\hat{\theta}']/\theta$
0.10	4.51
0.20	2.53
0.40	1.58
0.60	1.28
0.80	1.15
1.00	1.08

For example, suppose that the true number of recruits is around 40 and that the SE of the estimate is about 100. Now, $\theta/\sigma = 0.40$ and the mean of the censored estimate of births will be $1.58 \cdot 40 = 63.2$ for a positive bias of $63.2 - 40 = 23.2$ animals.

Appendix C. Likelihood Equation

Under the usual assumptions for capture–recapture studies (Pollock et al. 1990), the likelihood function can be partitioned into two components:

$$L = L_1 \times L_2$$

$$= P(\{u_i\}) \times P(\text{subsequent recaptures or losses} \mid \{u_i\}).$$

Seber (1982, p. 198) modeled L_1 conditional upon the number of animals *alive* in the population at sampling occasion i. The actual recruitment process between sampling occasion is not modeled. Consequently, it is difficult to impose a probability model that describes the recruitment process or to impose constraints to force all estimates of births to be nonnegative.

We follow, instead, the approach of Crosbie and Manly (1985) who conditioned upon the escapement and model L_1, the number of animals that enter the system between sampling occasions, as a multinomial distribution drawn from the escapement. Additionally, we follow the approach of Burnham (1991) who showed that L_2 can be written as the product of binomial distributions. The likelihood function is then the product of the following terms:

	Term	Distribution
(C.1)	$P(\{u_i; i = 1,\ldots,s\}\|N)$	Multinomial(N;$\{\psi_i^* p_i$; $i = 1,\ldots,s\}$)
(C.2)	$P(R_1\|n_1)$	Binomial(n_1;ν_1)
	$P(r_1\|R_1)$	Binomial(R_1;λ_1)
	$P(m_2\|T_2)$	Binomial(T_2;τ_2)
	$P(R_2\|n_1)$	Binomial(n_2;ν_2)
	$P(r_2\|R_2)$	Binomial(R_2;λ_2)
	$P(m_3\|T_3)$	Binomial(T_3;τ_3)
	⋮	⋮
	$P(m_{s-1}\|T_{s-1})$	Binomial(T_{s-1};τ_{s-1})
	$P(R_{s-1}\|n_{s-1})$	Binomial(n_{s-1};ν_{s-1})
	$P(r_{s-1}\|R_{s-1})$	Binomial(R_{s-1};λ_{s-1})

where b_i^* is the probability than an animal enters the system between sampling occasion i and $i + 1$, b_0^* refers to entrance before the first sampling occasion, $\psi_1 = b_0^*$, $\psi_{i+1} = \psi_i(1 - p_i)\phi_i + b_i^* h(\phi_{i-1})$, $h(\phi_{i-1})$ is the probability that an animal that enters between sampling occasion $i - 1$ and i survives to the next sampling occasion (for the case of uniform entry and constant mortality, $h(\phi_{i-1}) = (\phi_{i-1} - 1)/\log(\phi_{i-1})$), ν_i is the probability of a loss of capture at sampling time i, $i = 1,\ldots,s$, $\lambda_i = \phi_i(p_{i+1} + (1 - p_{i+1})\lambda_{i+1})$, $i = 1,\ldots,s - 1$, $\lambda_s = 0$, $\tau_i = p_i/(p_i + (1 - p_i)\lambda_i)$, $i = 1,\ldots,s$, $T_1 = R_1$, and $T_{i+1} = T_i - m_i + r_i = m_{i+1} + z_{i+1}$, $i = 2,\ldots,s$. There are $3s$ parameters, $\{\phi_i; i = 1,\ldots,s - 1\}$, $\{p_i; i = 1,\ldots,s\}$, and $\{b_i^*; i = 0,\ldots,s - 1\}$, and N subject to the constraint that $\Sigma b_i^* = 1$. This likelihood differs from Crosbie and Manly (1985) in that we allow losses on capture and express the second component, L_2 in a more convenient form. Losses on capture are assumed to be at random from animals captured and so are noninformative about $\{\phi_i\}$, $\{p_i\}$, $\{b_i^*\}$, or N. As in the Jolly–Seber model, not all parameters are individually identifiable. As in Crosbie and Manly (1985), MLEs obtained from this likelihood are identical to those from the Jolly–Seber model presented in Table 2 and also give rise to our equation (1). As shown by Burnham (1991), this representation is particularly convenient for deriving large-sample covariances. The large-sample variances and covariances for $\{\hat{\phi}_i\}$ and $\{\hat{p}_i\}$ are also the same as from the Jolly–Seber model, but the covariance formulae for $\{\hat{B}_i\}$ differ slightly, since we condition upon the escapement (N) rather than on the actual births ($\{B_i\}$). However, by using the formulae on conditional variances presented in Seber (1982, p. 9), identical covariances to those presented in Pollock et al. (1990) can be obtained from our results if suitable conditioning is performed.

This likelihood is not fully convenient for numerical optimization, since the parameter N appears in a factorial term. We further partition the component (C.1) of the likelihood into two parts:

(C.1a) = $P(u_.|N)$ which is binomial(N; $\Sigma\psi_i^* p_i$)

(C.1b) × $P(\{u_i\} \mid u_.)$ *which is multinomial*($u_.$; $\psi_i^* p_i/\Sigma\psi_i^* p_i$)

where $u_. = \Sigma u_i$ is the total number of unmarked animals seen. Now, the parameter N appears only in (C.1a). As shown by Sanathanan (1977), the conditional maximum likelihood estimates found by maximizing (C.1b) × (C.2) to find conditional MLEs for $\{\phi_i\}$, $\{p_i\}$, and $\{b_i^*\}$ and then using these conditional MLEs in (C.1a) to estimate N are asymptotically equivalent to the unconditional MLEs obtained by maximizing (C.1a) × (C.1b) × (C.2). Numerical optimization of the conditional MLE is relatively straightforward because no parameters appear in factorial term, the score functions (the first partial derivatives) are equivalent to those obtained from weighted least squares, and the expected information matrix can be obtained from the score functions. Good, stable, numerical methods to solve these type of equations are available (e.g., Press et al. 1989). By using the log-odds of the parameters, i.e., $\log(\theta/(1 - \theta))$, and the method of Lagrange multipliers, it is not difficult to constrain the estimates within the bounds of the parameter space, i.e., $0 \leq \{\hat{\phi}_i\}, \{\hat{p}_i\}$, and $\{b_i^*\} \leq 1$.

Appendix D. Asymptotic Variances and Covariances of Birth and Survival Terms

These formulae appear to differ from Crosbie and Manly (1985) because they used a different notation and did not consider losses on capture, and they differ from Pollock et al. (1990) because these are conditional upon the escapement rather than the actual number of births between the sampling occasions. All formulae are evaluated at the expected value of the statistics. When estimating the variances and covariances the actual value of the statistics is used unless constrained MLEs are used, in which case the expected value of the statistics should be estimated using the estimated parameters rather than from the observed values in the experiment.

$$\operatorname{cov}\{\hat{N}_2,\hat{N}_2\} = N_2(N_2 - n_2)\frac{M_2 - m_2 + R_2}{M_2}\left(\frac{1}{r_1} - \frac{1}{R_2}\right) + \frac{N_2 - M_2}{N_2 m_2} + N_2\frac{N - N_2}{N}$$

$$\operatorname{cov}\{\hat{N}_2,\hat{B}_2\} = N_2\phi_2(1 - p_2)\left(\frac{1}{r_2} - \frac{1}{R_2}\right)(N_2 - n_2 + R_2) + N_2\phi_2 - \frac{N_2 N_3}{N} - \operatorname{cov}\{\hat{N}_2,\hat{N}_2\}\phi_2$$

$$\operatorname{cov}\{\hat{N}_2,\hat{B}_j\} = \frac{-N_2 N_{j+1}}{N} + \frac{N_2 N_j \phi_j}{N} \qquad j = 3,\ldots,s - 2$$

$$\operatorname{cov}\{\hat{B}_i,\hat{B}_i\} = B_i^2(M_{i+1} - m_{i+1})\frac{M_{i+1} - m_{i+1} + R_{i+1}}{M_{i+1}^2} \times \left(\frac{1}{r_{i+1}} - \frac{1}{R_{i+1}}\right) + \frac{(M_i - m_i)}{(M_i - m_i + R_i)}\frac{[\phi_i R_i(N_i - M_i)]^2}{M_i^2} \times \left(\frac{1}{r_i} - \frac{1}{R_i}\right) + \frac{(N_i - n_i)(N_{i+1} - B_i)(N_i - M_i)(1 - \phi_i)}{N_i(M_i - m_i + R_i)}$$

$$+ \frac{(N_{i+1} - n_{i+1})(N_{i+1} - M_{i+1})}{m_{i+1}}$$

$$+ \frac{\phi_i^2 (N_i - n_i)(N_i - M_i)}{m_i}$$

$$+ \frac{B_i (N - B_i)}{N} \qquad i = 2, \ldots, s-2$$

$$\operatorname{cov}\{\hat{B}_i, \hat{B}_{i+1}\} = \frac{-\phi_{i+1}(N_{i+1} - n_{i+1})(N_{i+1} - M_{i+1})}{N_{i+1}}$$

$$\times \left\{ \frac{B_i R_{i+1}}{M_{i+1}} \left(\frac{1}{r_{i+1}} - \frac{1}{R_{i+1}} \right) + \frac{N_{i+1}}{m_{i+1}} \right\} - \frac{B_i B_{i+1}}{N}$$

$$i = 2, \ldots, s-3$$

$$\operatorname{cov}\{\hat{B}_i, \hat{B}_j\} = \frac{-B_i B_j}{N} \qquad i = 2, \ldots, s = 4, j > i+1$$

$$\operatorname{cov}\{\hat{\phi}_i, \hat{\phi}_i\}$$

$$= \phi_i^2 \left[\frac{(M_{i+1} - m_{i+1})(M_{i+1} - m_{i+1} + R_{i+1})}{M_{i+1}^2} \right.$$

$$\times \left(\frac{1}{r_{i+1}} - \frac{1}{R_{i+1}} \right) + \frac{(M_i - m_i)}{(M_i - m_i + R_i)} \left(\frac{1}{r_i} - \frac{1}{R_i} \right)$$

$$\left. + \frac{(1 - \phi_i)}{M_{i+1}} \right] i = 1, \ldots, s-2$$

$$\operatorname{cov}\{\hat{\phi}_i, \hat{\phi}_{i+1}\} = -\phi_i \phi_{i+1} \frac{(M_{i+1} - m_{i+1})}{M_{i+1}} \left(\frac{1}{r_{i+1}} - \frac{1}{R_{i+1}} \right)$$

$$i = 1, \ldots, s-3$$

$$\operatorname{cov}\{\hat{N}_2, \hat{\phi}_1\} = N_2 \phi_1 (1 - p_2)^2$$

$$\times \left[\frac{1}{r_2} - \frac{1}{R_2} + \frac{\left(1 - \frac{r_2}{R_2}\right)}{(r_1 - m_2)} \left(1 - \frac{1}{N_2}\right) \right]$$

$$\operatorname{cov}\{\hat{N}_2, \hat{\phi}_2\} = -N_2 \phi_2 (1 - p_2) \left(\frac{1}{r_2} - \frac{1}{R_2} \right)$$

$$\operatorname{cov}\{\hat{B}_i, \hat{\phi}_{i-1}\} = -\phi_{i-1} \phi_i (N_i - n_i) \left(1 - \frac{m_i}{n_i} \right)$$

$$\times \frac{(M_i - m_i + R_i)}{M_i} \left(\frac{1}{r_1} - \frac{1}{R_i} \right) \qquad i = 2, \ldots, s-2$$

$$\operatorname{cov}\{\hat{B}_i, \hat{\phi}_i\} = \phi_i \frac{(N_{i+1} - n_{i+1})}{N_{i+1}} \left(\frac{1}{r_{i+1}} - \frac{1}{R_{i+1}} \right)$$

$$\times \left[B_i \frac{(M_{i+1} - m_{i+1} + R_{i+1})}{M_{i+1}} + \phi_{i+2} (N_i - n_i) \right.$$

$$\left. \times \left(1 - \frac{m_i}{n_i} \right) \frac{r_{i+1}}{(z_{i+1} + m_{i+1})} \right] \qquad i = 2, \ldots, s-2$$

$$\operatorname{cov}\{\hat{B}_i, \hat{\phi}_{i+1}\} = -\phi_{i+1} (N_{i+1} - n_{i+1})$$

$$\times \left(1 - \frac{m_{i+1}}{n_{i+1}} \right) \left(\frac{1}{r_{i+1}} - \frac{1}{R_{i+1}} \right) \qquad i = 2, \ldots, s-3.$$

The variances and covariances of the estimated adjusted number of births are obtained by a Taylor-series expansion of the net births times the adjustment factor chosen. The variance of the estimated escapement is then the sum of the variances and covariances of the adjusted estimates.

Experimental Analysis of Microhabitat Selection by Juvenile Steelhead (*Oncorhynchus mykiss*) and Coho Salmon (*O. kisutch*) in a British Columbia Stream

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Replicate experiments were conducted in the Salmon River, British Columbia, during early summer 1990 to test the relative importance of velocity refuge, visual isolation, and overhead cover to microhabitat selection by steelhead (*Oncorhynchus mykiss*) parr and age-0 coho salmon (*O. kisutch*). Four types of artificial Plexiglas structures, the first three of identical construction, had different portions painted to provide increasing habitat complexity: velocity refuge alone, velocity refuge with visual isolation, all three features combined, and overhead cover alone. Steelhead parr selected structures with overhead cover alone or all three features significantly more often than those without overhead cover. Steelhead also selected structures adjacent to the swiftest velocities available and closest to other natural overhead cover, which accounted for most differences in use of the same structure in different locations. In contrast, few age-0 coho salmon used any structures. Those that did selected the three types of structures with velocity refuge about equally, but significantly more often than those with overhead cover alone, regardless of their location. Field experiments such as this hold promise for elucidating mechanisms of habitat selection by stream salmonids.

Des expériences répétées ont été effectuées dans la rivière Salmon, en Colombie-Britannique, au début de l'été 1990; elles avaient pour objet de tester l'importance relative des refuges contre le courant rapide, de l'isolation visuelle et du couvert pour le choix du microhabitat par des tacons de truite arc-en-ciel (*Oncorhynchus mykiss*) anadrome et par des cohos (*O. kisutch*) d'âge 0. Nous avons construit quatre types de structures artificielles en Plexiglas, dont les trois premières étaient identiques, et nous avons peint des parties différentes pour augmenter la complexité de l'habitat : refuge contre le courant seulement, refuge avec isolation visuelle, les trois caractéristiques combinées et le couvert seulement. Les tacons de truite ont choisi les structures avec couvert seulement ou avec les trois caractéristiques de façon nettement plus fréquente que celles qui n'avaient pas de couvert. Les truites ont aussi choisi les structures proches des courants les plus rapides et aussi les plus proches du couvert naturel, ce qui représentait la plupart des différences dans l'utilisation de la même structure en des lieux différents. Par contre, les saumons cohos d'âge 0 ont été peu nombreux à utiliser les structures. Ceux qui l'ont fait ont choisi de façon à peu près égale les trois types de structures avec refuge contre le courant, mais toutefois de façon nettement plus fréquente que celles qui offraient seulement un couvert, quel que soit leur emplacement. Les travaux de terrain comme celui-ci sont prometteurs pour l'étude des mécanismes du choix de l'habitat par les salmonidés dans les cours d'eau.

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Most of what has been discovered about the positions that salmonids hold in streams has been learned by observation. For example, early research showed that juvenile chinook salmon (*Oncorhynchus tshawytscha*) and steelhead (*O. mykiss*) undergo ontogenetic shifts in habitat selection, moving to faster and deeper water as they grow larger (Chapman and Bjornn 1969; Everest and Chapman 1972). Investigators have also described much about the average characteristics of microhabitat that salmonids use. Adult brown trout (*Salmo trutta*) are known to select shaded positions with low velocity (Gosse and Helm 1981; Bachman 1984; Clapp et al. 1990) whereas rainbow trout select relatively open positions in swifter currents (Cunjak and Green 1983; Baltz et al. 1991).

However, these observations give relatively little insight into why stream salmonids select certain positions over others. In contrast, more has been learned about these mechanisms by experimental manipulations, often under controlled conditions. Research during the last 25 yr has shown that salmonids seek optimum conditions of depth and velocity for foraging, within the constraints of intraspecific and interspecific dominance hierarchies (Jenkins 1969; Fausch 1984; Fausch and White 1986; Hughes and Dill 1990; Hughes 1992a, 1992b). Moreover, stream fish must do all of this and not get eaten, i.e., they must also weigh the risk of predation (Dill and Fraser 1984; Power 1987; Lima and Dill 1990; Brown and Moyle 1991).

The experiments done to date suggest that physical structures forming habitat for stream salmonids provide three features relevant to the trade-offs among foraging, intraspecific and interspecific competition, and predation risk. These features are velocity refuge, visual isolation, and overhead cover (Campbell and Neuner 1985). Structures that afford *velocity refuge* allow fish to hold low-velocity positions adjacent to swifter currents and may thereby maximize their energy intake from drifting invertebrates while minimizing the cost of swimming to main-

tain position (Mundie 1969; Everest and Chapman 1972; Fausch 1984; but see Hughes and Dill 1990 for an alternative view). Structures that provide *visual isolation* from other fish can reduce agonistic behavior within intraspecific and interspecific dominance hierarchies and may thereby increase the density of fish that can occupy a given area (Kalleberg 1958; Mesick 1988). Lastly, structures that provide *overhead cover* afford hiding places and decrease visibility of salmonids to terrestrial and aquatic predators by reducing light beneath the structures, both of which are thought to reduce predation risk (Gibson and Power 1975; Helfman 1981).

Here I report the results of field experiments designed to test the relative importance of these three features to microhabitat selection by steelhead parr and coho salmon (*Oncorhynchus kisutch*) fry in a British Columbia stream. Most experiments aimed at discovering the relative importance of one or more of the features to stream salmonids have been done in the laboratory (Hartman 1963; Wilzbach 1985; Mesick 1988) or with fish that were constrained in outdoor channels (Butler and Hawthorne 1968; Baldes and Vincent 1969; DeVore and White 1978; McMahon and Hartman 1989), so the results may, to some degree, have been artifacts of handling or constraining fish. Two recent field experiments provided evidence that salmonids select certain conditions, but were unable to fully separate their relative importance due to constraints of the experimental design (Shirvell 1990) or because only one of the three features was manipulated (Cunjak and Power 1987).

Study Area

Two replicate experiments were conducted in separate 250-m reaches of the Salmon River, a productive, moderate-gradient tributary (~0.4% in the reach studied; McMynn and Vernon 1954) of the lower Fraser River in southwestern British Columbia. Fish distributions and habitat were described by Hartman (1965, 1968) and Hartman and Gill (1968). The two study reaches were located about 500 m apart, 2 km downstream from a site sampled by Hartman (1965, S-13 in his fig. 6). The river here consisted primarily of riffles and runs with cobble and gravel substrate and pools formed by large woody debris originating from the second-growth riparian forest.

Juvenile coho salmon and steelhead and a few juvenile and adult cutthroat trout (*Oncorhynchus clarki*) occurred in the reaches studied. Hartman (1968) found trout (steelhead and cutthroat combined) to age 3 based on scale annuli, most of which were <160 mm fork length (FL). Age-0 coho salmon captured by seining (3-mm-mesh) in the downstream study reach on 2 July 1990 averaged 59.5 mm total length (TL) (SE = 0.60, range 42–82 mm, $n = 162$). The age-0 steelhead captured were <60 mm TL and age-1 (hereafter parr) were >80 mm TL, based on a length–frequency histogram ($n = 93$).

Four runs in each study reach were chosen for the two experiments because Facchin and Slaney (1977) found the highest density of steelhead parr in this habitat type. The runs averaged 4.4 m wide (range 2.5–6.1 m) and 13 m long (range 4.5–32 m). They had mean depths of 28–49 cm (maxima 33–57 cm), mean substrate diameters of 2–7 cm (maxima 6–13 cm), and mean velocities of 12–32 cm·s^{-1} (maxima 35–65 cm·s^{-1}). Water temperature ranged between 13.0 and 15.0°C during the first experiment (28 June to 6 July 1990) and between 13.5 and 17.5°C during the second (11–19 July 1990). Discharge was low and stable at both sites during experiments (downstream reach: 0.16 m^3·s^{-1}; upstream reach: 0.11 m^3·s^{-1}). Underwater visibility averaged 5.1 m (range 4.2–6.3 m, $n = 10$). It was determined daily by measuring the distance through water at which a 13 × 18 cm white diving slate disappeared from view.

Methods

Design and Layout of Experimental Structures

The replicate experiments consisted of setting out artificial habitat structures that afforded fish different combinations of velocity refuge, visual isolation, and overhead cover and recording which ones were used by fish. The structures were constructed of three pieces of 6-mm-thick Plexiglas, two of which formed a T-shaped structure when viewed from above (Fig. 1). The T structure was 10 cm wide and 20 cm long and was buried so that 5 cm protruded above the substrate. The third piece was a 20 × 30 cm overhead cover centered above the T structure and suspended 10 cm above the bed on 6-mm-diameter steel legs.

Three different treatments were constructed by painting portions of the structures black. Unpainted structures provided only velocity refuge (hereafter treatment VR). However, because I suspected that clear structures might be difficult for fish to see when submerged, a 0.5-cm-wide black strip was painted along the edges of the leading piece of the T structure to provide a visual reference (Fig. 1). When the entire T was painted black the structure afforded velocity refuge and visual isolation (hereafter VR + VI). When all pieces were painted black the structure provided velocity refuge, visual isolation, and overhead cover (hereafter ALL). Each of these three treatments was hydraulically identical to avoid bias caused by altering flow patterns when adding features.

Pilot research conducted in mid-June indicated that some steelhead parr and coho presmolts selected overhead cover alone. Therefore, I designed a fourth treatment having a simple visual reference of the same dimensions as the leading piece of the T structure, placed beneath a black overhead cover identical to other treatments. The visual reference was a wicket (i.e., an inverted U) of 3-mm steel rod that was 10 cm wide, buried so that 5 cm protruded above the bed. This treatment afforded only overhead cover (hereafter OHC) with minimal velocity refuge. On three occasions, coho fry held positions directly downstream from a leg supporting an overhead cover, presumably using it as a velocity refuge (cf. Sutterlin and Waddy 1975), but neither species was ever observed to hold a position directly downstream from the wicket rod.

A set of four structures, one of each treatment, was set out in a 1 × 4 m plot in each of the four runs selected for each experiment. Runs in each study reach that had the highest steelhead parr densities were selected by underwater observation. Age-0 coho were abundant throughout. Plots were oriented with the long axis parallel to stream flow, and the boundaries were marked on the bed with nylon lines. In the first experiment, structures were placed in a line down the center of the long axis of the plot with their centers positioned 0.90 m apart and 0.65 m from the upstream and downstream plot boundaries. In the second experiment, structures were offset 10 cm from the centerline on alternate sides (Fig. 1) to minimize flow disturbance for adjacent upstream and downstream structures. Two runs used in the second experiment could not accommodate 1 × 4 m plots, so shorter, wider plots of 4 m^2 total area (2.7 × 1.5 m and 3 × 1.3 m) were used

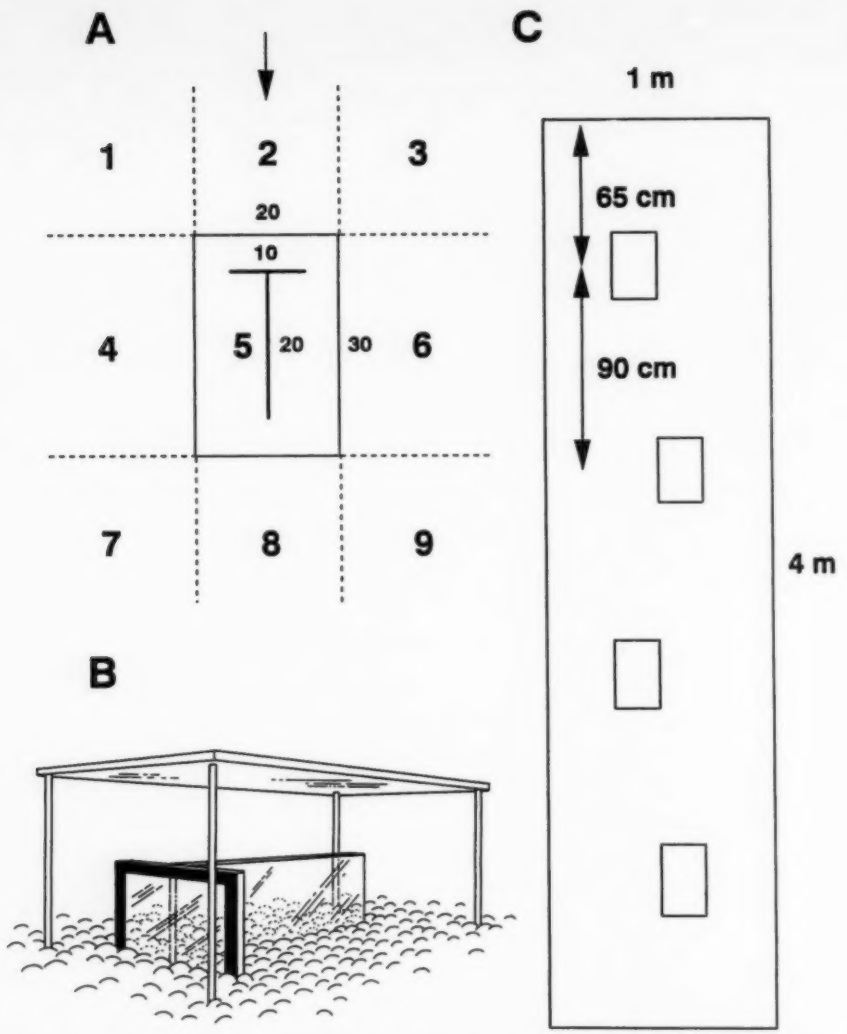

FIG. 1. Design and layout of structures used in experiments. (A) Top view showing orientation of structures to flow direction (arrow) and quadrats 1–9 in which fish were counted. Small numbers show dimensions (cm). (B) Three-dimensional side view of VR structure showing clear overhead cover and T structure with black stripe to provide a visual reference. The T structure is 5 cm high, and the overhead cover is supported 10 cm above the bed. (C) Layout of four structures in a 1 × 4 m plot in experiment 2. Structures were offset to the left and right of the midline to minimize disturbance to flow near adjacent structures.

instead. Complex channel morphology caused flow to change direction midway through the longer plot, so structures were arrayed down the centerline but oriented with their long axis parallel to local flow direction. In the shorter plot, structures were alternated along two lines parallel to flow that were spaced 0.7 m apart. The structures in each line were placed with their centers 0.9 m apart, and the edges of the most upstream and downstream structures were 0.3 m from the two adjacent plot boundaries.

Physical Measurements

The effects of treatments on the physical environment were measured during the last 2 d of observation of experiment 2. Light above and beneath structures was measured with a Li-Cor model 185B meter and 192S underwater quantum sensor. Light was measured approximately 10 cm above, 2 cm beneath, and 5 cm beneath (for black overhead covers only) the center of each overhead cover immediately after fish counts were completed for each plot. On one day, light was also measured at positions of 10 steelhead parr (two or three per plot) that were beneath natural instream overhead cover or in the shade created by terrestrial vegetation. All fish were inside, or within 50 cm of, the plot boundaries.

Water velocity was measured upstream and to the sides of one T structure that was positioned near one of the plots in water 15 cm deep with uniform flow. Velocity was measured

(± 0.5 cm·s^{-1}) using a Novonic Streamflo meter (Nixon Instrumentation Ltd., UK) that consisted of a 1-cm-diameter propeller on a thin wand. Four replicate velocity measurements (10-s integration) were made 2.5 cm above the bed of 1- to 2-cm gravel at each of 13 marked locations before and after the structure was carefully removed.

Water depths, water velocities, and substrate diameters were measured at each structure location the day before the last day of observation of each experiment (see below). Depths were measured 10 cm from each edge of the overhead cover (i.e., at locations of the numbers 2, 4, 6, and 8 in Fig. 1A). Water velocity 2 cm above the bed (hereafter bottom velocity) was measured with a Marsh-McBirney model 201 electromagnetic flow meter, 15 cm to the right and left of the leading corners of the overhead cover. A substrate particle was randomly selected from each of the thirty-six 0.3×0.3 m quadrats within each plot and its second-longest dimension measured as an index of mean diameter. Surface turbulence above each structure was scored in three classes: none (surface not rippled), slight (surface rippled but structure visible), and moderate (structure barely visible due to surface turbulence). I also measured the distance from each structure to the nearest natural overhead cover capable of concealing a 15-cm fish from overhead view, such as undercut banks and submerged logs, rootwads, or dense brush.

Observation Protocol

The number of coho fry and steelhead parr that were using each structure and the total number in each plot were counted at four 2-d intervals from the beginning of each 8-d experiment. Total counts of fish in each plot were also made immediately before structures were first placed. The fish were counted by underwater observation using a mask, snorkel, and wetsuit. I entered the water 10–30 m downstream from each plot and carefully moved upstream until the downstream plot boundary was sighted. I recorded the location of each fish in the plot as it came into view at the limits of underwater visibility (4–6 m). Many of the largest steelhead parr held positions away from natural or artificial overhead cover and were observed long before they became frightened and moved, which suggests that observations of habitat use were unbiased.

Each fish position was mapped on an underwater slate, using the structures as points of reference. Locations of fish beneath or within 20 cm of the edge of the overhead cover for each structure were recorded in nine quadrats (Fig. 1). Lengths of steelhead parr were estimated within 25-mm size classes by comparing fish with substrate or structure features and later measuring the distances (cf. Fausch and White 1981). On two occasions during experiment 2, a cutthroat trout was observed using a structure. These data were included because the size, behavior and habitat selection of juvenile cutthroat observed throughout the reach were indistinguishable from those of steelhead parr.

Many fish, especially coho fry, that were not directly beneath structures nevertheless used one or more features afforded by them. For example, some fish held positions 10–20 cm upstream from structures providing velocity refuge, alone or with other features (i.e., treatments VR, VR + VI, or ALL) and were observed to use the structures as velocity refuges only. I defined structures as being used by fish if any of the features they afforded were used, based on the following criteria. Fish were judged to be using velocity refuge features only if their head was positioned within 20 cm directly upstream or downstream from a T structure and their tailbeat frequency was substantially lower than in adjacent positions with ambient velocity. The difference in tailbeat frequency often could be observed when fish rose gradually in the water column to inspect drifting invertebrates and subsequently sank back to their focal point near the stream bed. Fish were recorded as using visual isolation features only if their body was within 5 cm upstream or to one side of a black T structure and within 2 cm of the bed, so that they were obscured from view by conspecifics on opposite sides of the structure. Fish were judged to be using overhead cover only if more than half their body length was beneath a black overhead cover, or in the shade provided by it. Only two fish, one coho and one steelhead, were observed doing the latter. All fish that were using all three features were directly beneath ALL treatment structures. Coho salmon that held positions >10 cm above the stream bed, above or within 20 cm of a structure (i.e., floaters; Puckett and Dill 1985), were not counted as using them.

The four different types of structures were placed in random order in each of the four plots and randomly permuted after each day of observation according to a balanced set of Latin Squares unique to each experiment. This design ensured that each treatment occurred in a different upstream-to-downstream position in each run (or block) during every 2-d period and was randomly permuted to every position and run combination only once during the experiment (Gill 1978).

In addition to randomizing treatments within and among runs, I started observations at a different randomly selected run each day to control for bias due to time of day. Runs were observed in upstream order to avoid effects of increased turbidity on downstream visibility and fish behavior. After completing work in the most upstream run, I waited about 1 h to allow any turbidity to clear before beginning observations on the downstream run. Structures were cleaned of any periphyton and moved to their new locations soon after observations and measurements were completed for each plot. The work required about 7 h (~09:00–16:00) on each day of observation.

To determine whether the frequent disturbance of moving structures might bias the results, I set out an additional ALL structure in the center of a 1-m^2 "control" plot located within each experimental reach. This plot was observed at the same 2-d intervals as the others but was left undisturbed throughout the experiment.

Statistical Analysis

Fish response to structures was analyzed at three hierarchical levels: fish near structures (i.e., in quadrats 1–9, beneath or within 20 cm of the overhead cover), fish using structures (as defined above), and the specific features of structures used by fish (i.e., velocity refuge, visual isolation, overhead cover). Because at most two steelhead parr or coho fry were observed using any one structure simultaneously, data for the second level of analysis were nearly binary (i.e., the structure was used or not) rather than normally distributed, which precluded using analysis of variance based on the Latin Squares design (Gill 1978). Instead I used logistic regression (Hosmer and Lemeshow 1989), which is specifically designed to analyze effects of categorical and continuous variables on binomial response variables such as the proportion of structures used by fish. Separate logistic regressions were calculated for steelhead parr and coho fry, first based on five experimental factors (experiment,

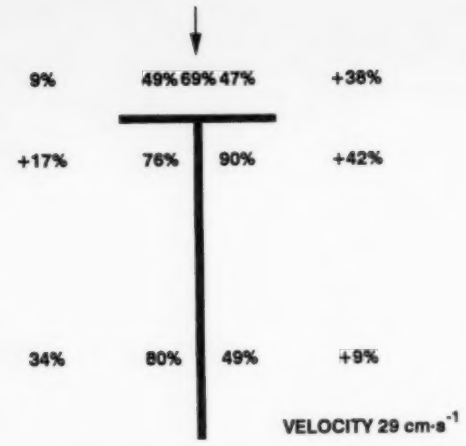

FIG. 2. Percentage velocity reduction around a T structure, based on measurements at 13 points before and after removing the structure. Percentage velocity increases are indicated by plus signs. Measurement points were oriented in three lines, 2.5 cm upstream and 2.5 cm downstream from the leading edge of the T and 5 cm upstream from the downstream end. The seven points closest to the T were all 2.5 cm from all Plexiglas surfaces and from each other. The six measurements to the right and left were arranged along lines parallel to flow that were 5 cm from the leading corners of the T. Ambient velocity of 29 cm·s^{-1} was measured at the junction of the two pieces, after the structure was removed.

day of observation, treatment, run, position within run) and then using five physical habitat variables (depth, velocity, substrate, distance to cover, surface turbulence). Although the logistic regression (unlike the balanced Latin Squares design) could not fully account for repeated measures effects, such as might be caused by a particular fish that selected overhead cover every day, it could detect other block and position-within-block effects that might prove to be important.

Results

Effect of Structures on Light and Water Velocity

The overhead covers greatly reduced light beneath them when painted black and had little effect when clear. Black overhead covers reduced ambient light (i.e., that measured above the structures) by an average of 99.8% (SE = 0.094, n = 18) 2 cm beneath the structure ($P < 0.0001$ by paired t-test after arcsine square-root transformation for percentages) and 99.1% (SE = 0.22, n = 18) 5 cm beneath the structure (i.e., 5 cm above the stream bed) ($P < 0.0001$). In contrast, light 2 cm beneath clear overhead covers was not significantly different from ambient light ($\bar{x}$ = 0.5% higher beneath structures, SE = 13.5, n = 16, P = 0.66) but varied greatly in some cases due to shadows that affected only one measurement. Light above all types of structures averaged 4100 lx (SE = 847, range 360 – 18 900, n = 34) and averaged 4.6 lx (SE = 1.03, range 0–11, n = 18) 2 cm beneath black overhead covers, when measured during the last 2 d of experiment 2. Light measured at positions of 10 steelhead parr beneath natural overhead cover, or in the shade of overhanging vegetation, averaged 198 lx (SE = 228, range 0–556).

Measurements upstream, downstream, and on each side of the leading piece of a T structure showed that a velocity of 29 cm·s^{-1} was reduced an average of 66% (range 47–90%) within 2.5 cm upstream and within 2.5 cm of the longitudinal piece downstream (Fig. 2). In contrast, water velocity was up to 42% higher 5 cm to the sides of the leading piece, most likely because flow was shunted around it. Thus, measurements of light and velocity around structures indicated that the velocity refuge and overhead cover features had the desired effect on the physical environment.

Fish near Structures

Experimental plots had high average densities of both steelhead parr (experiment 1: $\bar{x}$ = 0.9·m^{-2}; experiment 2: $\bar{x}$ = 1.3·m^{-2}) and coho fry (experiment 1: $\bar{x}$ = 4.6·m^{-2}; experiment 2: $\bar{x}$ = 5.6·m^{-2}) when structures were present. However, there was no indication that fish were attracted into plots by the structures because about the same numbers were present before and after the structures were placed. Single counts of steelhead parr made immediately before structures were placed were always within the range of those made when structures were present ($\bar{x}$ = 4.4 parr per plot, SE = 0.5) for all eight plots used in both experiments. For coho fry, this was true for four of eight plots ($\bar{x}$ = 20.7 parr per plot, SE = 1.2). For two of the other four plots, counts made before structures were placed were two fish less than the lowest count with structures present, but in the other two plots, counts made before structures were placed were one and four fish more than the highest counts afterwards. Overall, these data suggest that few coho were attracted into the plots by structures.

The most general hypothesis to test about fish response to structures is whether fish in plots chose positions near structures over those in the surrounding natural habitat. For example, fish might display a general thigmotactic response to structures (cf. Hartman 1963; DeVore and White 1978) or derive benefit from velocity refuges or proximity to escape cover. If so, one would expect a higher density of fish beneath and within 20 cm of the structures (i.e., in quadrats 1–9; Fig. 1) than the average for the plot as a whole. The density of coho fry near structures was not significantly different from that in the whole plot (P = 0.22 and P = 0.37 for the two experiments by paired t-test; n = 16 each), suggesting that coho were not generally attracted to positions near structures. Density of steelhead parr was significantly greater in quadrats 1–9 than in the whole plot in experiment 1 (P = 0.01) but not in experiment 2 (P = 0.49). However, failure to reject the null hypothesis of no general preference for positions near structures does not preclude the possibility that fish used certain locations or types of structures more than others. For example, density of steelhead was significantly higher in the strip upstream, beneath, and downstream from structures (i.e., in quadrats 2, 5, and 8) than in adjacent strips to the right and left in both experiments ($P \leq 0.0002$ by paired t-test for both). Moreover, because most (76%) steelhead in quadrats 1–9 were either upstream or beneath structures, and the majority (63%) were also using the structures (as defined above), only relationships between steelhead using structures and the five experimental factors were analyzed by logistic regression. A similar analysis was conducted for coho.

Use of Structures by Steelhead

Steelhead parr used structures with overhead cover and all three features significantly more often than structures with velocity refuges alone or combined with visual isolation (Fig. 3;

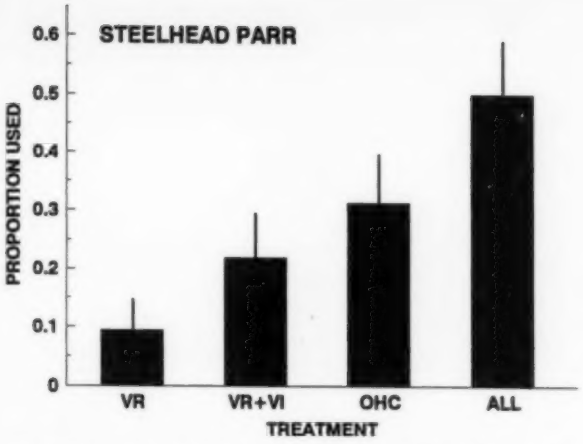

FIG. 3. Proportion of structures of four different treatment combinations that were used by steelhead parr. Vertical lines show +1 SE. Treatments were velocity refuge (VR), velocity refuge and visual isolation (VR + VI), overhead cover (OHC), and all three features combined (ALL).

$P = 0.0009$ for contrast of VR and VR + VI versus OHC and ALL treatments). However, the effects of visual isolation added to velocity refuge (VR versus VR + VI, $P = 0.15$) and of velocity refuge and visual isolation added to overhead cover (OHC versus ALL, $P = 0.086$) were not significant. These three a posteriori contrasts were judged significant at $P = 0.017$ to control the experimentwise error rate at $P = 0.05$.

Logistic regression indicated that, of the five experimental factors tested (experiment, day, treatment, run, position), only treatment, run, and position accounted for significant variation in the proportion of structures used ($P < 0.015$ for likelihood-ratio (LR) chi-square tests of models with individual variables versus intercept only). Moreover, a model with these three variables accounted for no less variation than one with all five ($P = 0.79$ by LR chi-square) and was the most parsimonious based on Akaike's Information Criterion (AIC; Akaike 1985). None of the two-way interactions between treatment and other main effects (e.g., experiment-by-treatment) were significant ($P > 0.13$ by LR chi-square for interactions added to the five-variable model). Interactions with run and position were not considered further because different runs were used in each experiment.

The previous analysis indicated that use of structures by steelhead parr depended not only on the type of structure (treatment), but also on its location (i.e., run and position). I therefore used logistic regression to determine whether any of the five physical habitat variables (depth, average bottom velocity, diameter of substrate beneath structures, surface turbulence, distance to nearest natural overhead cover) could account for differences due to structure location. After accounting for the differential effect of treatments, the proportion of structures used was significantly related only to velocity and distance to nearest cover ($P < 0.007$ by LR chi-square for both versus model with treatment alone), which themselves were not significantly correlated ($r = -0.171, P > 0.05, n = 128$). However, because the relationships were not logistic, especially for distance to cover, these continuous variables were transformed to categorical by dividing the observations into four sets of about equal sample size each (Fig. 4) as suggested by Hosmer and

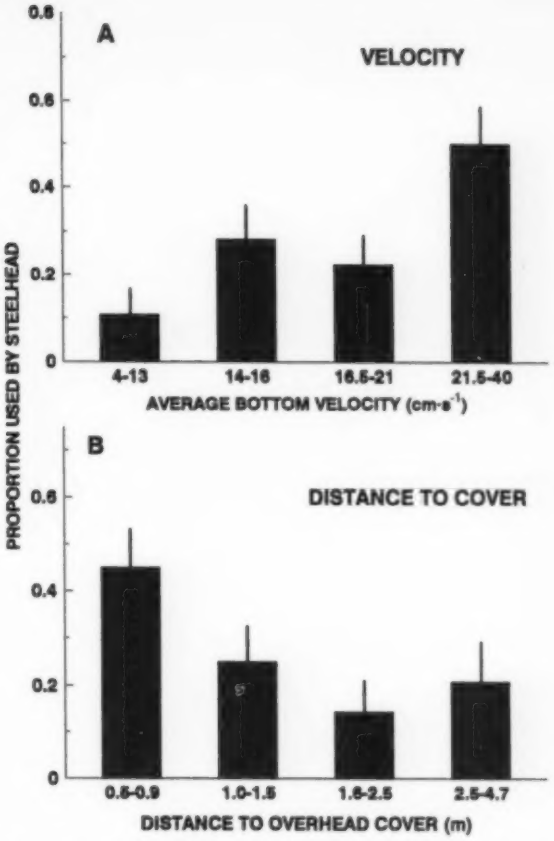

FIG. 4. Proportion of structures used by steelhead parr, divided into four classes by (A) average bottom velocity and (B) distance to nearest natural overhead cover. Vertical lines denote +1 SE. Bottom velocity was measured 2 cm above the bed and 15 cm to the right and left of the leading edge of each overhead cover. Natural overhead cover included undercut banks and submerged logs, rootwads, and dense brush.

Lemeshow (1989). The resulting regression, which included treatment, velocity, and distance to cover, accounted for about as much information (AIC = 135.7) as the former one containing treatment, run, and position (AIC = 132.5), indicating that the physical habitat variables accounted for most of the variation in structure use due to different locations. Single a posteriori contrasts showed that steelhead selected structures located adjacent to swifter velocities (>21 cm·s^{-1}, $P = 0.003$) and within 0.9 m of natural overhead cover ($P = 0.02$) significantly more often than those in lower velocities or farther from cover.

The disturbance of moving structures every 2 d probably had little effect on their use by steelhead, based on observations of the ALL structure in the 1-m^2 "control" plot. In experiment 1, no steelhead parr ever colonized the structure, probably due to low ambient velocity, whereas in experiment 2, one or two parr were observed beneath it on each observation day. In neither case, therefore, was there any change during the experiment. On one occasion in each experiment the number of steelhead in a plot was counted twice, at 3- and 5-h intervals, and on another day counts were made 4 h apart in a control plot.

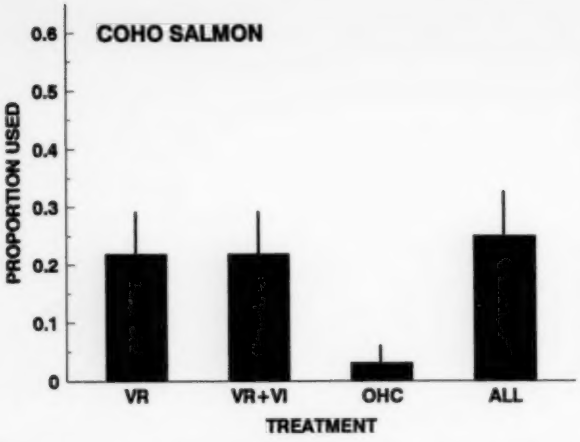

FIG. 5. Proportion of structures of four different treatment combinations used by age-0 coho salmon. Vertical lines denote + 1 SE. Treatment combinations are as in Fig. 3.

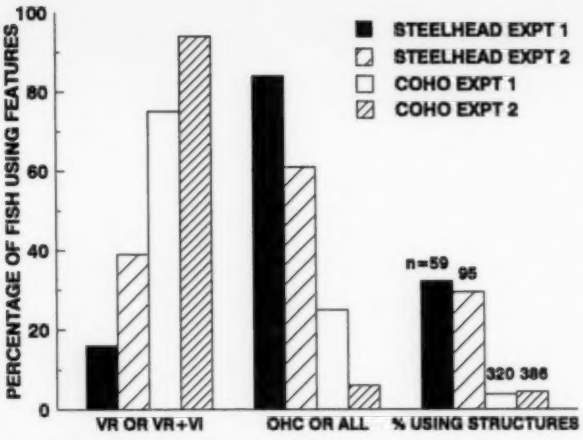

FIG. 6. Percentage of the steelhead parr and coho salmon fry occupying structures in experimental and control plots that were using velocity refuge features, with or without visual isolation, versus those using overhead cover or all three features. The set of bars at the right shows the percentage of all fish counted in plots that were using any structure.

In all three cases the number of parr in the plot and the number in quadrats 1–9 near structures varied by only one fish or were identical for the two counts, suggesting that time of day also had little effect on the results.

Although a few age-0 steelhead were observed in the plots, none were ever beneath structures or used any of the features they provided. Densities were low ($\bar{x}$ = 1.2 per plot, SE = 0.2, range 1–5 per plot, n = 32), probably because this age class generally occupies riffles during the first months after emergence (Hartman 1965).

Use of Structures by Coho

Age-0 coho used the three structure types that had velocity refuges (VR, VR + VI, and ALL) significantly more often than structures with overhead cover alone (P = 0.029 by a posteriori contrast; Fig. 5). Of the five experimental factors tested by logistic regression, only treatment and day of observation accounted for significant variation ($P < 0.04$ by LR chi-square for each versus the model with intercept alone). Moreover, a model with treatment and day accounted for no less variation than other more complex models with three to five main effects (P = 0.67 or greater by LR chi-square) and was the most parsimonious based on AIC. There were no significant interactions between treatment and other variables.

The effect of day was significant because, for both experiments combined, only one age-0 coho was observed using any structure on the first experiment day compared with six to nine coho on subsequent days. This might lead one to suspect that use of structures by coho increased through time. However, use of the ALL structures in control plots did not change during either experiment (no coho were observed using them), and the number of coho in control plots did not increase with time. Counts on successive days were 5, 5, 6, 7, and 6 for experiment 1 (two counts on day 2) and 4, 4, 4, and 3 for experiment 2.

The proportion of structures used by coho was not significantly related to any of the five physical habitat variables, after accounting for the effects of treatment ($P > 0.1$ for all by LR chi-square). This was expected because neither the effects of run nor those of position were significant in the logistic regression based on experimental factors alone.

Features Used by Fish

Analysis of the specific features that steelhead and coho used corroborated results on the types of structures fish used. In both experiments, a high percentage of the steelhead parr that occupied structures (including those in control plots) used overhead cover or all three features (70% for both experiments, n = 47; Fig. 6), rather than velocity refuge alone or in combination with visual isolation. All steelhead using overhead cover or all three features were directly beneath structures, except for one fish positioned adjacent to an overhead cover in the shade it provided. In contrast, all but one of those using velocity refuge features, with or without visual isolation, held positions directly upstream from structures. In five cases, two steelhead parr were observed simultaneously using opposite sides of the black T beneath a structure having all three features. In one other case, two steelhead were observed simultaneously using a structure providing overhead cover alone.

Nearly the opposite was found for coho fry. On average, 86% of those using structures (n = 29) selected the velocity refuge feature alone (none used visual isolation). Only one of these fish, and three others using overhead cover alone or all three features, held positions directly beneath structures. Of all fish observed in experimental and control plots, about a third of the steelhead parr occupied structures (30 and 32%) whereas very few of the coho observed used structures (4% in both experiments). Most of the steelhead using structures with overhead cover (OHC of ALL treatments) were among the smaller parr observed in the plots, although the proportion was not significantly different from expected (29 of 33 using overhead cover were <150 mm versus 116 of 154 observed in plots; P = 0.10 by G-test).

Most steelhead parr that were not using structures held positions beneath or within about 30 cm of natural overhead cover located near the plot boundaries or in shade from overhanging terrestrial vegetation. For example, one plot was crossed by a 10-cm-diameter branch suspended about 10 cm above the bed, beneath which two to four steelhead parr held positions during each observation.

Discussion

Steelhead Parr

During early summer, steelhead parr selected artificial habitat structures that provided overhead cover significantly more often than those with velocity refuge alone or with visual isolation (Fig. 3). Physical measurements confirmed that the structures having all three features reduced light to low levels and substantially decreased water velocity (Fig. 2), and their design ensured that fish holding positions close to opposite sides of the same structure were visually isolated.

The experiments indicated that, of the three features, overhead cover and the low light it provided was the most important relative to the others. Shirvell (1990) also inferred from his field experiment using simulated rootwads that steelhead parr selected positions in a coastal British Columbia stream primarily in response to low light levels. However, although I found that overhead cover was the primary feature selected, the data also suggest that both velocity refuge and visual isolation contributed to use of structures by parr. For example, 30% of the steelhead using structures in both experiments selected velocity refuge features alone, or combined with visual isolation, and all but one of these held positions directly upstream from structures. Moreover, 21% of the parr using structures were visually isolated from a conspecific positioned on the opposite side of a black T, beneath a structure with all three features. Experiments by other investigators have also shown that various other stream salmonids selected locations beneath or within the most complex cover available, especially during winter (Hartman 1963; DeVore and White 1978; McMahon and Hartman 1989).

Other physical features adjacent to the structures also were important predictors of steelhead parr use. Steelhead selected structures that were adjacent to the swiftest velocities in the plots (21–40 $cm \cdot s^{-1}$; Fig. 4). Such velocity refuges adjacent to relatively swift currents that provide abundant drifting invertebrates may allow fish to optimize foraging (Fausch 1984). Steelhead also selected structures that were within about 1 m of other pieces of natural overhead cover such as undercut banks and submerged logs, rootwads, or dense brush. Moreover, most of the steelhead parr observed in the plots that were not using structures (69% on average) held positions adjacent to or beneath this natural overhead cover, or in other shaded positions, suggesting that steelhead selected positions with light below some threshold.

Facchin and Slaney (1977) found significant positive correlations between steelhead parr density and various size classes of boulders (>30 cm in diameter) in riffles of another Fraser River tributary nearby. They observed parr using positions downstream from boulders in turbulent water of riffles and the upstream portions of runs and surmised that the fish were selecting locations that afforded velocity refuges and overhead cover. Like Shirvell (1990), I suspect that complex structure provides different functions under different circumstances. For example, based on Facchin and Slaney's (1977) results, steelhead parr might have used the velocity refuge or visual isolation features of structures that lacked overhead cover (i.e., VR and VR + VI) more had they been placed in riffles, whereas in runs with little surface turbulence the overhead cover feature was most important. Surface turbulence was, at most, moderate and varied relatively little among structures, which may be the reason that it accounted for little variation in structure use by steelhead.

Neither day of experiment nor time of day noticeably affected fish use of structures. Numbers of steelhead and coho using permanent ALL structures in control plots were unchanged during both experiments, and replicate counts of steelhead in a few plots revealed little change between about 09:00 and 16:00. Numbers of steelhead and coho in plots also changed little before and after structures were placed, indicating that few fish were attracted into plots by the structures. These observations suggest that results were not an artifact of gradual acclimation to structures or of diurnally shifting preferences of fish for structures.

Coho Fry

In contrast with steelhead parr, only a small proportion of the age-0 coho in the plots used any structure (4% in both experiments). Those that did selected the three treatments that provided velocity refuge (i.e., VR, VR + VI, and ALL) significantly more often than the one with overhead cover alone (Fig. 5), and most used the velocity refuge feature of structures they occupied (Fig. 6). Shirvell (1990) also concluded that coho fry of similar size, and studied during the same months, selected positions primarily based on low velocity, regardless of whether this required moving to deeper or shallower positions when stream flow was purposely manipulated. Mundie (1969) found that simple current deflectors greatly increased the number of coho fry that remained in an experimental outdoor channel with swift ambient velocity, compared with two channels without deflectors that had swift or slow average velocity. Together, these results suggest that velocity refuges are the most important feature selected by age-0 coho during early summer.

Much of the reason that so few age-0 coho, which averaged about 60 mm TL, used structures may be that velocity refuges and visual isolation were generally plentiful in the cobble and large gravel substrate that was abundant in most plots. In addition to coho that defended velocity refuges near the stream bed in regions of the plot with faster velocity, there were many other "floaters" (Puckett and Dill 1985) that milled about in the water column in regions with slower velocity, regardless of their proximity to structures or other natural cover. Mundie (1969) proposed that in swift velocities where invertebrate drift is unidirectional, larger coho defend individual positions whereas in lower velocities, coho live in loose aggregations and scramble for food, as has been observed by others (cf. Fausch and White 1986; C.S. Shirvell, Pacific Biological Station, Nanaimo, B.C. V9R 5K6, personal communication).

Differences reported here in microhabitat selected by coho fry and steelhead parr are supported by the findings of other investigators (e.g. Hartman 1965; Bisson et al. 1988). Bisson et al. (1988) proposed that these differences were related to body and fin morphology, coho being better adapted to hold low-velocity positions and steelhead better adapted for swifter currents. However, coho also undergo ontogenetic and seasonal shifts in habitat use, and so may have used structures more had experiments been conducted at another time. For example, complex structure that provides overhead cover, velocity refuge, and visual isolation has been shown to be important for coho during winter (Bustard and Narver 1975), especially during freshets (Tschaplinski and Hartman 1983; Swales et al. 1986; McMahon and Hartman 1989), and during the spring seaward migration as smolts (McMahon and Holtby 1992).

Implications for Management and Further Research

The results reported here will be useful for fishery biologists throughout the Pacific Northwest who attempt to enhance hab-

itat for steelhead parr (e.g., Ward and Slaney 1979, 1981). Some may argue that use of certain microhabitat features by steelhead during early summer does not substantiate their importance for survival because bottlenecks that limit populations may occur during other seasons, especially winter (Bustard and Narver 1975). However, while this may be true, it is unlikely that microhabitat requirements during summer are generally unimportant to stream salmonids. Instead, the two are probably linked to varying degrees in different populations. For example, survival of juvenile salmonids that overwinter in streams may strongly depend on body growth during the previous summer, as Hunt (1969) found for brook trout (*Salvelinus fontinalis*) in Lawrence Creek, Wisconsin. Similarly, ocean survival of anadromous salmonids has been shown to increase with body weight at smolting (Bilton et al. 1982; Ward et al. 1989). Thus, survival, and ultimately fitness, may be closely related to body growth during summer, which in turn depends on quality of foraging positions, constraints of the dominance hierarchy, and trade-offs with predation risk.

Observational data on microhabitat use of stream salmonids are prevalent in the literature (e.g., Murphy et al. 1989; Heggenes et al. 1991a, 1991b). These data are widely used to construct models for predicting how man's perturbations of flow and channel structure affect the amount of suitable habitat for fish and, by inference, fish abundance (Bovee 1986; Fausch et al. 1988). However, unanticipated correlations and interactions among variables may render models based on observations alone misleading in many cases and ultimately produce poor predictions of how stream salmonids will respond to perturbations. Although such observations have provided empirical evidence suggesting which microhabitat features are important to stream salmonids, these data should now be used to generate critical hypotheses for experimentation, preferably in the field. We have much to learn from replicated field manipulations of habitat features, even based on simple experiments like those described here (see also Angermeier and Karr 1984). Such field experiments, and others in more controlled environments where investigators focus on closely simulating the natural environment (e.g., McMahon and Hartman 1989), hold promise for elucidating mechanisms of habitat selection by stream salmonids on which sound management of lotic ecosystems can be based.

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Fixed-Location Hydroacoustic Monitoring Designs for Estimating Fish Passage Using Stratified Random and Systematic Sampling

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Five alternative finite sampling designs are compared using 15 d of 24-h continuous hydroacoustic data to identify the most favorable approach to fixed-location hydroacoustic monitoring of salmonid outmigrants. Four alternative approaches to systematic sampling are compared among themselves and with stratified random sampling (STRS). Stratifying systematic sampling (STSYS) on a daily basis is found to reduce sampling error in multiday monitoring studies. Although sampling precision was predictable with varying levels of effort in STRS, neither magnitude nor direction of change in precision was predictable when effort was varied in systematic sampling (SYS). Furthermore, modifying systematic sampling to include replicated (e.g., nested) sampling (RSYS) is further shown to provide unbiased point and variance estimates as does STRS. Numerous short sampling intervals (e.g., 12 samples of 1-min duration per hour) must be monitored hourly using RSYS to provide efficient, unbiased point and interval estimates. For equal levels of effort, STRS outperformed all variations of SYS examined. Parametric approaches to confidence interval estimates are found to be superior to nonparametric interval estimates (i.e., bootstrap and jackknife) in estimating total fish passage.

Nous comparons cinq types d'échantillonnage de taille finie en utilisant des données hydroacoustiques recueillies en continu pendant 15 jours pour repérer la méthode la plus favorable à la surveillance hydroacoustique fixe des salmonidés en avalaison. Quatre différentes méthodes d'échantillonnage systématique sont comparées entre elles et à l'échantillonnage aléatoire stratifié (STRS). Nous avons observé qu'une stratification de l'échantillonnage systématique (STSYS) sur une base quotidienne réduit l'erreur d'échantillonnage dans les travaux de surveillance sur plusieurs jours. La précision de l'échantillonnage était prévisible selon les niveaux variables d'effort du STRS, mais il n'était pas possible de prévoir l'ampleur ni le sens du changement de précision lorsque l'effort variait dans l'échantillonnage systématique (SYS). En outre, nous confirmons que la modification de l'échantillonnage systématique par inclusion d'échantillons répétés (p. ex. à plusieurs degrés) (RSYS) fournit des estimations ponctuelles et des estimations de variantes non biaisées, comme c'est le cas pour le STRS. Il est nécessaire d'établir de nombreux intervalles courts d'échantillonnage (p. ex. 12 échantillons d'une durée de 1 min par heure) avec surveillance heure par heure à l'aide du RSYS pour obtenir des estimations efficaces et non biaisées pour les points et les intervalles. Pour des niveaux égaux d'effort, le STRS dépassait toutes les variantes du SYS examinées. Les approches paramétriques pour les estimations des intervalles de confiance apparaissent supérieures aux estimations non paramétriques (c.-à-d. bootstrap et jackknife) pour estimer le passage total des poissons.

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Balancing water demands of fisheries, hydroelectric production, and irrigation on the Columbia and Snake rivers has resulted in considerable effort to restore and enhance salmon and steelhead runs. These efforts include installation of in-turbine diversion screens, bypass facilities, and the use of spillwater at hydroelectric projects in order to increase downstream survival of salmon and steelhead smolts. Other activities include monitoring the timing and magnitude of spring outmigrants both within and between seasons. Hydroacoustic sampling at hydroelectric facilities is an important tool in estimating fish passage to evaluate various bypass methods at dams. Because management decisions rely on hydroacoustic passage estimates, it is important that sampling methods provide reliable point estimates and associated error variances. The purpose of this paper is to describe the application of finite sampling techniques to fixed-location hydroacoustic monitoring in order to provide valid point and interval estimates of fish passage.

Sources of uncertainty associated with hydroacoustic passage indices include (1) measurement error intrinsic to the hydroacoustic sampling process and (2) sampling error associated with sampling the spatial and temporal dimensions of smolt passage through a hydroelectric facility. Classical finite sampling theory (Hansen et al. 1953; Cochran 1977) can be used to estimate fish passage from hydroacoustic data and incorporate these error sources. The theory is particularly well suited to this task because the sampling methods can provide unbiased point estimates of total passage and of their variance without requiring distributional assumptions concerning the

nature of the dispersion pattern of fish passage or assumptions of independence. The only requirement is that the spatial and temporal dimensions of fish passage be sampled probabilistically. This can be accomplished through simple random sampling (SRS) or stratified random sampling (STRS) of the fish passage at a dam. We use the term "fish passage" loosely. In the context of finite sampling theory, the sampling unit is the time interval during which fish pass the transducer within a passage way. The response variable measured is then the "weighted number of fish" that estimates the total number of fish within that time–space cell. The response variable is not the individual fish counts.

Currently, fixed-location hydroacoustic sampling at hydroelectric facilities is based almost exclusively on systematic sampling (SYS). In SYS, every kth unit in a population is sampled. This method divides the survey durations into evenly spaced time intervals where every kth inteval is sampled. For example, let our target population be a 24-h period of fish passage and let our sampling interval be 3 min. In this example, fish passage refers to the number of fish going through a passage route during a 3-min time interval. If we sample every fifth time interval (3 min out of every 15 min) throughout the day, there is a choice of $K = 5$ systematic samples that can be drawn. Because the entire day is systematically sampled, selection of the first time interval determines the whole sample. However, systematic designs pose several problems. The most troublesome is that no consistent estimate of variance exists unless replicate systematic samples can be taken (Cochran 1977). Williamson (1982) discussed the problem of variance estimation from midwater hydroacoustic surveys using systematic samples from transect surveys. In the absence of consistent variance estimators, both Cochran (1977) and Wolter (1984) proposed a number of alternate variance estimators for systematic samples. Through a simulation study, Wolter (1984) found that the preferred variance estimator depended on whether the data exhibited a linear trend, a stratification effect, or autocorrelation. Because we believe that stratification effects exist in fish passage, we used Monte Carlo simulations to compare alternative variance estimators and determine the validity of statistical inference on fish passage using SYS.

Confidence intervals explicitly express the degree of precision associated with parameter estimates. By providing upper and lower bounds on fish passage, confidence intervals can also be used to compare passage estimates associated with different bypass alternatives and between seasons. Although the finite sampling theory provides point and variance estimators that are nonparametric, confidence interval estimation typically requires distributional assumptions (i.e., normal theory). Both parametric and nonparametric approaches to interval estimation in conjunction with finite sampling theory estimates are compared to determine the most appropriate interval estimators with nominal coverage and minimal width.

This paper presents modifications of classical SYS (Cochran 1977, p. 205–232) which addresses the problems of biased variance estimation and reduced precision with SYS. The proposed solutions are addressed within the context of existing methodology for hydroacoustic monitoring. Performance of SYS methods is then compared with STRS. Specific objectives of this paper are as follows: (1) determine the relative advantages of stratified systematic sampling (STSYS) over SYS in multiday monitoring programs, (2) determine the statistical properties and advantages of using replicated systematic sampling (RSYS) over SYS, (3) determine the relative advantages of STRS over alternative SYS designs, and (4) identify the most appropriate approaches to confidence interval estimation in conjunction with fixed-location hydroacoustic monitoring of fish passage. It is anticipated that these results will improve the statistical defensibility and precision of hydroacoustic monitoring and provide guidance on future improvements in sampling techniques.

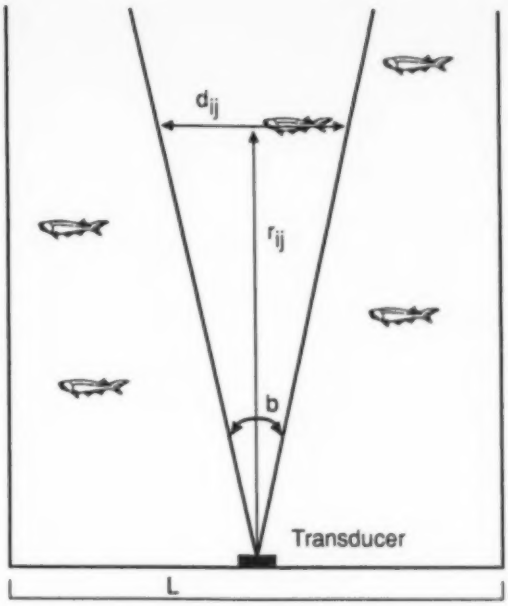

FIG. 1. Schematic of the cross-sectional area sampled by an acoustic beam within a turbine intake of width L. Fish detected are weighted as a function (1) of the cross-sectional width b of the acoustic beam and the range r of each detection to account for the sampling fraction at different distances from the transducer.

Methods

Hydroacoustic Sampling Technique

The hydroacoustic data used in this study were collected at both the Rock Island and Rocky Reach dams (Raemhild et al. 1988; Steig et al. 1988; Steig and Ransom 1989) located 730 and 761 km upriver from the mouth of the Columbia River, respectively. There were 15 data sets collected in 1988 and 1989, three from Rock Island and 12 from Rocky Reach. Each data set consisted of a 24-h continuous hydroacoustic survey of fish passage. The data were collected by transducers placed at the turbine intakes upstream of the trashrack. The transducers at both Rock Island and Rocky Reach dams were wall mounted and faced downward. The transducers were oriented to sample fish as they passed through the ensonified volumes and entered the turbine intake.

Fish traces from chart recorder echograms were entered into computer storage. The data consisted of the following information for each fish detected by the transducer: (1) date of detection, (2) time the fish entered the beam, (3) time the fish exited the beam, (4) distance from the transducer when the fish entered the beam, and (5) distance from the transducer when the fish exited the beam.

Individual fish detections are transformed into "weighted number of fish" and summed to estimate the total number of fish passing into the turbine intake during each sampling interval. Weighting is necessary because the cross-section of the acoustic beam is triangular in shape, while the intake the fish are passing through is rectangular in shape (Fig. 1). Because the acoustic beam does not give complete coverage of the intake, each fish detection is weighted to account for this sampling fraction. The weighted number of fish ($\hat{y}_i$) in a sampling interval is calculated according to the formula

$$(1)\quad \hat{y}_i = \sum_{j=1}^{n_i} \frac{L}{2r_{ij} \tan\left(\frac{b}{2}\right)}$$

where L = turbine intake width, r_{ij} = average distance of the jth fish detected in the ith sampling interval from the transducer = (entrance range + exit range)/2, b = transducer beam width expressed in degrees, and n_i = number of fish detected in the ith sampling interval. Hence, the weighting is simply expanding the diameter of the acoustic beam at the range of each detected fish to the width of the passage (i.e., $2r_{ij} \tan(b/2)$ is the beam diameter).

Each of the fifteen 24-h data sets constituted a target population with known numbers of weighted fish passage along with temporal variance that were used to compute the expected value and true variance in fish passage estimates under various sampling schemes. Monte Carlo simulation studies sampled these temporal data in continuous time to investigate the statistical properties of alternative estimators of total fish passage.

Hydroacoustic Measurement Error

Consider a sampling design based on sampling fish passage at a turbine intake or bypass slot on an hour-by-hour basis. In other words, the target population is the fish passage within a "unit hour." Within that hour, only a fraction of time will be sampled by the hydroacoustic apparatus. For example, let the hour be divided into N equal time intervals of length $60/N$ min. Then, if a random sample of n of these N intervals is hydroacoustically monitored, an unbiased estimate of total fish passage (Y) during that unit hour is

$$\hat{Y} = \frac{N}{n} \sum_{i=1}^{n} \hat{y}_i$$

where $\hat{y}_i$ = number of fish estimated to have passed through the unit during the ith interval by the hydroacoustic apparatus (i.e., weighted number of fish), provided $\hat{y}_i$ is an unbiased estimate of y_i, the actual fish passage in the ith interval.

The variance of $\hat{Y}$ will consist of both sampling error and hydroacoustic measurement error. To see how these error sources contribute to the overall variance, we can use the total variance formula

$$\begin{aligned}\mathrm{Var}(\hat{Y}) &= \mathrm{Var}_{y_i}\left[E\left(\hat{Y} \mid y_i\right)\right] + E_{y_i}\left[\mathrm{Var}\left(\hat{Y} \mid y_i\right)\right] \\ &= \mathrm{Var}_{y_i}\left[E\left(\frac{N}{n}\sum_{i=1}^{n} \hat{y}_i \mid y_i\right)\right] \\ &\quad + E_{y_i}\left[\mathrm{Var}\left(\frac{N}{n}\sum_{i=1}^{n} \hat{y}_i \mid y_i\right)\right] \\ &= \mathrm{Var}_{y_i}\left[\frac{N}{n}\sum_{i=1}^{n} y_i\right] \\ &\quad + E_{y_i}\left[\frac{N^2}{n^2}\sum_{i=1}^{n} \mathrm{Var}\left(\hat{y}_i \mid y_i\right)\right] \\ &= \frac{N^2}{n}\left(1 - \frac{n}{N}\right) s_{y_i}^2 + \frac{N^2}{n}\overline{\mathrm{Var}\left(\hat{y}_i \mid y_i\right)} \end{aligned} \quad (2)$$

where

$$s_{y_i}^2 = \frac{\sum_{i=1}^{N} (y_i - \bar{Y})^2}{N - 1}$$

and the average hydroacoustic measurement error is

$$\overline{\mathrm{Var}\left(\hat{y}_i \mid y_i\right)} = \frac{1}{N}\sum_{i=1}^{N} \mathrm{Var}\left(\hat{y}_i \mid y_i\right) .$$

In a sample survey of fish passage, Var ($\hat{Y}$) would be estimated by

$$(3)\quad \hat{\mathrm{Var}}\left(\hat{Y}\right) = \frac{N^2}{n}\left(1 - \frac{n}{N}\right) s_{\hat{y}_i}^2$$

where

$$s_{\hat{y}_i}^2 = \frac{\sum_{i=1}^{n} (\hat{y}_i - \bar{\hat{y}})^2}{(n - 1)}$$

$$\bar{\hat{y}} = \frac{\sum_{i=1}^{n} \hat{y}_i}{n} .$$

This variance estimate (3) of total fish passage has the expected value

$$\begin{aligned}(4)\quad E\left[\frac{N^2}{n}\left(1 - \frac{n}{N}\right) s_{\hat{y}_i}^2\right] &= \frac{N^2}{n}\left(1 - \frac{n}{N}\right) E\left(s_{\hat{y}_i}^2\right) \\ &= \frac{N^2}{n}\left(1 - \frac{n}{N}\right) s_{y_i}^2 + \frac{N^2}{n}\left(1 - \frac{n}{N}\right)\overline{\mathrm{Var}\left(\hat{y}_i \mid y_i\right)} .\end{aligned}$$

Hence, the empirical variance, $s_{\hat{y}_i}^2$, implicitly incorporates both sampling error and hydroacoustic measurement error. Comparison of variance formulas (2) and (4) shows, however, that variance estimator (3) is negatively biased. The second term in formula (4) has the additional factor $(1 - n/N)$ that variance (2) does not possess. Typically, it is believed that the measurement error (i.e., $\overline{\mathrm{Var}\left(\hat{y}_i \mid y_i\right)}$) is small relative to $s_{\hat{y}_i}^2$, so this bias is anticipated to be small. Furthermore, the bias is proportional to $(1 - n/N)$, and this factor will be close to 1 in many applications. A conservative but always valid estimator of the true variance (2) can be obtained by ignoring the finite population correction (i.e., $(1 - n/N)$) in variance estimator (3).

These computations show that we can avoid explicit measurement of hydroacoustic measurement error and still incorporate that error source in the overall variance of fish passage by considering formula for sampling error alone. Measurement error in this context refers to random sources of errors and not to systematic errors (i.e., calibration problems) that will manifest themselves as bias in point estimation. Note, the bias of

order ($1 - n/N$) in variance expression (4) vanishes for infinite sampling theory as applied in regression analysis or ANOVA.

Hydroacoustic Sampling Error

The survey models presented below represent sampling designs for estimating total fish passage over time at a single fish passage route through a dam. To estimate total passage at a hydroelectric project, stratification or two-stage sampling is necessary to combine estimates from multiple routes throughout a dam. Because the principles of temporal sampling of outmigration are the same for one or multiple passage routes, the single route case is chosen for illustration.

Stratified Random Sampling (STRS)

In estimating fish passage at a turbine intake, an hour serves as a natural stratum for two reasons: (1) dam operations typically change on the hour, so any corresponding changes in fish passage within the dam will also occur on the hour, and (2) hours are small time units, flexible enough to accommodate the diel patterns of outmigration rates.

Using hours as strata, total fish passage is defined as

$$Y_{ST} = \sum_{h=1}^{H} \sum_{i=1}^{N} y_{hi} = \sum_{h=1}^{H} N\bar{Y}_h$$

and is estimated by

$$(5) \quad \hat{Y}_{ST} = \sum_{h=1}^{H} N \frac{\sum_{i=1}^{n} \hat{y}_{hi}}{n} = \sum_{h=1}^{H} N\bar{\hat{y}}_h$$

where H = number of hours sampled, N = number of time intervals in each hour (e.g., if we choose to sample 10-min intervals, then $N = 6$), n = number of time intervals randomly sampled each hour, and $\hat{y}_{hi}$ = sum of the weighted fish detections during time interval i ($i = 1, \ldots, N$) in hour h ($h = 1, \ldots, H$) and

$$\bar{\hat{Y}}_h = \frac{\sum_{i=1}^{N} y_{hi}}{N}$$

and

$$\bar{\hat{y}}_h = \frac{\sum_{i=1}^{n} \hat{y}_{hi}}{n}.$$

Variance of the passage estimate is an unknown quantity that also needs to be estimated. By sampling each stratum (i.e., hour) independently of the others, we can sum the variance estimates over strata to obtain an unbiased estimate of variance of total fish passage in STRS. The variance to be estimated is

$$(6) \quad \mathrm{Var}(\hat{Y}_{ST}) = \sum_{h=1}^{H} N^2 \left(1 - \frac{n}{N}\right) \frac{s_h^2}{n}$$

where

$$s_h^2 = \frac{\sum_{i=1}^{N} (y_{hi} - \bar{\hat{Y}}_h)^2}{N - 1}$$

and is estimated by

$$(7) \quad \hat{\mathrm{Var}}(\hat{Y}_{ST}) = \sum_{h=1}^{H} N^2 \left(1 - \frac{n}{N}\right) \frac{s_h^2}{n}$$

where

$$s_h^2 = \frac{\sum_{i=1}^{n} (\hat{y}_{hi} - \bar{\hat{y}}_h)^2}{n - 1}.$$

Both the point estimate (5) and variance estimate (7) are unbiased in STRS.

Systematic Sampling (SYS)

The advantage of using SYS in hydroacoustic monitoring is in logistical ease of selecting a starting point in time and repeating the sampling on a regular basis throughout the survey. Typically, smpling is conducted at least hourly, often multiple times per hour over the course of the survey. For SYS to provide an unbiased point estimate, the time frame (T) must be a whole number multiple of the sampling interval (I) and sample frequency (m) such that

$$K = \frac{T}{Im}$$

where I = duration of a single sampling interval, K = number of systematic samples that can be drawn (i.e., integer values only), m = number of time intervals hydroacoustically monitored in each systematic sample over time T, and T = total survey duration. For example, during a survey duration of $T = 60$ min, $K = 6$ different schematic examples could be drawn if $m = 2$ time intervals of $I = 5$ min each are monitored.

For SYS, total fish passage is defined as

$$Y_{SYS} = \sum_{i=1}^{K} \sum_{j=1}^{m} y_{ij} = m \sum_{i=1}^{K} \bar{y}_i$$

and is estimated by

$$(8) \quad \hat{Y}_{SYS(i)} = K \sum_{j=1}^{m} \hat{y}_{ij} = mK\bar{\hat{y}}_i, i = 1, \ldots, K$$

where y_{ij} = sum of the weighted fish detections during time interval j ($j = 1, \ldots, m$) in the ith possible systematic sample ($i = 1, \ldots, K$). Note that each of the possible K systematic samples yields an unbiased estimate of Y_{SYS}.

The variance of $\hat{Y}_{SYS(i)}$ is

$$(9) \quad \mathrm{Var}(\hat{Y}_{SYS(i)}) = Km[(Km - 1)S_{SRS}^2 - K(m-1)S_{WSY}^2]$$

where S_{SRS}^2 = simple random sample variance among the y_{ij}, given by

$$\frac{\sum_{i=1}^{K} \sum_{j=1}^{m} (y_{ij} - \bar{Y})^2}{Km - 1}$$

and S_{WSY}^2 = variance of the y_{ij} within systematic samples given by

$$\frac{\sum_{i=1}^{K} \sum_{j=1}^{m} (y_{ij} - \bar{y}_i)^2}{K(m - 1)}$$

where

$$\bar{Y} = \frac{\sum_{i=1}^{K}\sum_{j=1}^{m} y_{ij}}{Km}$$

and

$$\bar{y}_i = \frac{\sum_{j=1}^{m} y_{ij}}{m}.$$

Formulas (5–9) for STRS and SYS, as presented here for estimating fish passage, are well known in survey sampling literature (e.g., Cochran 1977).

In the absence of replication, there is *no unbiased* variance estimator for fish passage using systematic sampling (Cochran 1977). Wolter (1984) proposed eight alternative variance estimators and concluded that the performance of the estimators depends on the underlying distributional properties of the data. We considered four variance estimators for SYS of fish passage, three recommended by Cochran (1977, p. 224–226) and Wolter (1984) and $\hat{\text{Var}}(\bar{Y}_{ST})$. The three estimators recommended by Wolter (1984) in his investigation are as follows:

$$(10)\quad \hat{V}_1 = (Km)^2\left(1 - \frac{1}{K}\right)\frac{s_1^2}{m}$$

where

$$s_i^2 = \frac{\sum_{j=1}^{m}(y_{ij} - \bar{y}_i)^2}{m - 1}$$

$$(11)\quad \hat{V}_2 = (Km)^2\left(1 - \frac{1}{K}\right)\frac{s_2^2}{m}$$

where

$$s_2^2 = \frac{\sum_{j=2}^{m}(y_{ij} - y_{i,j-1})^2}{2(m - 1)}$$

$$(12)\quad \hat{V}_3 = (Km)^2\left(1 - \frac{1}{K}\right)\frac{s_3^2}{m}$$

where

$$s_3^2 = \frac{\sum_{j=3}^{m}(y_{ij} - 2y_{i,j-1} + y_{i,j-2})^2}{6(m - 2)}.$$

The fourth estimator comes from STRS, but since we sampled the data using SYS, it will be relabeled as

$$(13)\quad \hat{V}_4 = \sum_{h=1}^{H} N^2\left(1 - \frac{n}{N}\right)\frac{s_h^2}{n}$$

where

$$s_h^2 = \frac{\sum_{i=1}^{n}(y_{ij} - \bar{y}_i)^2}{n - 1}.$$

In computing $\hat{\text{Var}}(\bar{Y}_{ST})$ on the systematic samples, we treated the data as if they had been sampled via the stratified method.

Stratified Systematic Sampling (STSYS)

During multiday monitoring programs, the variance component S_{SRS}^2 can be anticipated to increase as the variations in fish counts include within-hour, between-hours, and perhaps, between-seasons variation. To correct for the loss of precision in multiday systematic samples, daily stratification can be used to block on day effects and at the same time maintain the logistical advantage of SYS. Stratified systematic sampling can be readily implementable because personnel typically check the hydroacoustic equipment daily. During the daily inspection, a new random starting point for the SYS could be selected, resulting in STSYS on a daily basis. The STSYS estimator of total fish passage is then

$$(14)\quad \hat{Y}_{STSYS(i)} = K\sum_{l=1}^{D}\sum_{j=1}^{m} y_{ijl}$$

which is the sum of the daily SYS estimates (i.e., $\hat{Y}_l$, $l = 1, \ldots, D$) over the D days of monitoring. The stratified systematic sampling estimator is an unbiased estimator with associated variance

$$(15)\quad \text{Var}(\bar{Y}_{STSYS}) = \sum_{l=1}^{D}\{Km[(Km - 1)S_{SRS,l}^2 - K(m - 1)S_{WSY,l}^2]\}$$

where

$$S_{SRS,l}^2 = \frac{\sum_{i=1}^{K}\sum_{j=1}^{m}(y_{ijl} - \bar{Y}_l)}{(Km - 1)}$$

$$S_{WSY,l}^2 = \frac{\sum_{i=1}^{K}\sum_{j=1}^{m}(y_{ijl} - \bar{y}_{il})^2}{K(m - 1)}$$

and where

$$\bar{Y}_l = \frac{\sum_{i=1}^{K}\sum_{j=1}^{m} y_{ijl}}{Km}$$

$$\bar{y}_{il} = \frac{\sum_{j=1}^{m} y_{ijl}}{m}.$$

Although (14) provides an unbiased point estimate, no consistent or unbiased variance estimator for (15) exists.

Replicated Systematic Sampling (RSYS)

Systematic sampling of fish passage through time, although logistically desirable, does not provide a consistent or unbiased variance estimate. Alternatively, RSYS provides the opportunity to retain logistical simplicity while yielding unbiased point and variance estimates. The method of replicated sampling requires very few essential steps, yet provides desirable properties through the principles of replication and randomization (Kish 1965, p. 127–132).

The general steps for RSYS are as follows:

(1) a systematic sample is drawn that provides an unbiased estimate of fish passage $\hat{Y}_i$; let I denote the length of the sampling interval (minutes) and n the number of intervals sampled per

TABLE 1. Comparison of the sampling variances (i.e., Var($\hat{Y}$)) for five alternative sampling schemes (SYS, RSYS, STRS, STSYS, and STRSYS) for various allocations of effort when In = 12 min using multiday continuous hydroacoustic monitoring data (l = number of systematic samples, I = length of sampling interval, n = number of sampling intervals each hour).

Data set[a]	Sampling scheme n/l[b] or	$l/n/I$[c]	Var($\hat{Y}$) SYS	RSYS	STRS	STSYS	STRSYS
1							
RO521	2/6	2/1/6	138 273	702 880	339 295	399 664	393 321
RO522	3/4	3/1/4	714 454	484 045	305 615	480 595	354 366
RO524	4/3	4/1/3	317 595	565 102	292 749	146 171	386 023
RO526	6/2	6/1/2	952 105	536 257	261 712	628 472	360 470
	12/1	12/1/1	562 706	371 135	219 060	263 813	264 648
2							
R513	2/6	2/1/6	3 192 284	4 837 632	340 872	1 019 811	4 898 646
R514	3/4	3/1/4	3 779 105	3 705 984	259 696	2 365 551	3 698 584
R520	4/3	4/1/3	1 067 417	2 918 112	217 185	467 161	3 025 633
R521	6/2	6/1/2	881 961	2 424 544	172 859	600 654	2 352 138
	12/1	12/1/1	632 077	1 526 720	126 971	432 122	1 480 822
3							
RO521	2/6	2/1/6	1 928 258	1 619 360	763 439	742 485	1 023 592
RO522	3/4	3/1/4	1 006 734	1 334 293	640 819	468 474	959 318
R513	4/3	4/1/3	539 316	1 276 000	557 084	326 485	864 856
R514	6/2	6/1/2	562 814	1 012 491	472 967	295 927	706 335
	12/1	12/1/1	792 794	766 059	383 353	428 123	583 919
4							
RO521	2/6	2/1/6	2 086 279	7 060 464	746 989	644 956	4 238 267
RO522	3/4	3/1/4	5 075 216	5 064 800	626 262	2 431 113	3 164 591
R520	4/3	4/1/3	388 314	3 969 936	557 626	295 302	2 499 776
R521	6/2	6/1/2	986 946	2 925 936	483 637	707 322	1 993 953
	12/1	12/1/1	583 880	1 636 741	383 854	341 347	1 159 522
5							
RO524	2/6	2/1/6	1 932 374	1 868 992	745 038	774 519	1 053 699
RO526	3/4	3/1/4	622 014	1 220 373	595 661	415 033	888 179
R513	4/3	4/1/3	507 634	1 533 856	548 631	318 030	911 879
R514	6/2	6/1/2	1 455 824	1 039 787	445 313	521 797	718 655
	12/1	12/1/1	405 540	804 469	359 021	354 588	585 947
6							
RO524	2/6	2/1/6	1 937 334	5 645 192	728 588	676 990	4 268 375
RO526	3/4	3/1/4	4 438 046	4 132 960	581 114	2 377 672	3 093 631
R520	4/3	4/1/3	275 846	3 403 496	549 173	286 847	2 546 799
R521	6/2	6/1/2	1 196 904	2 382 267	455 983	933 202	2 006 273
	12/1	12/1/1	388 424	1 408 184	359 522	267 812	1 161 551
Average			1 311 616	2 272 602	450 670	680 401	1 721 459

[a]File codes identify Rock Island Dam in 1988 (IO), Rocky Reach Dam in 1988 (RO) and 1989 (R), and date of sampling (e.g., 521: 21 October).
[b]Sampling effort for SYS, STRS, and STSYS.
[c]Sampling effort for RSYS and STRSYS.

hour; (2) the number of unique systematic samples that can be drawn is $60/(In) = K$; values of I and n are chosen such that K is an integer, (3) a random selection of l of K possible systematic samples is drawn yielding l unbiased estimates of total fish passage $\hat{Y}_i$ for $i = 1, \ldots, l$; (4) the RSYS estimate of fish passage is then the average of the individual point estimates where

$$(16)\quad \hat{Y}_{\text{RSYS}} = \sum_{i=1}^{l} \frac{\hat{Y}_i}{l}$$

with variance

$$\text{Var}(\hat{Y}_{\text{RSYS}}) = \frac{\left(1 - \frac{In}{60}\right)}{l} \frac{\sum_{i=1}^{K} (\hat{Y}_i - Y)^2}{(K-1)}$$

and variance estimator

$$(17)\quad \hat{\text{Var}}(\hat{Y}_{\text{RSYS}}) = \frac{\left(1 - \frac{In}{60}\right)}{l(l-1)} \sum_{i=1}^{l} (\hat{Y}_i - \hat{Y}_{\text{RSYS}})^2 .$$

As long as the individual $\hat{Y}_i$ are unbiased (systematic sampling assures this property), $\hat{Y}_{\text{RSYS}}$ will be unbiased. Furthermore, variance estimator (17) is an unbiased estimator of $\text{Var}(\hat{Y}_{\text{RSYS}})$ and can be used in confidence interval construction. Because current hydroacoustic sampling procedures are based on SYS, RSYS retains the logistical ease of existing technology.

TABLE 2. Average confidence interval coverage (%) for 95% confidence intervals and widths (parentheses) for STRS of 12 min/h averaged over the 15 data sets. For each data set 1000 Monte Carlo simulations were performed (i.e., total of 15 000 simulations).

Interval estimator[a]	*n/l*				
	2/6	3/4	4/3	6/2	12/1
Z	92.3	93.1	93.0	94.0	94.3
	(1123)	(1049)	(1007)	(941)	(862)
log	92.8	93.6	93.7	94.4	94.7
	(1133)	(1057)	(1014)	(947)	(867)
Expanded log	93.2	94.1	94.1	94.8	95.0
	(1158)	(1075)	(1032)	(961)	(878)
Square root	92.4	93.4	93.4	94.2	94.5
	(1123)	(1049)	(1007)	(941)	(862)
Bootstrap	84.9	90.9	92.6	94.1	95.4
	(877)	(956)	(977)	(971)	(941)
Nonparametric bootstrap	84.3	90.4	92.2	93.8	95.2
	(866)	(950)	(973)	(967)	(937)
Bootstrap (RE)	94.6	97.5	98.2	98.7	99.2
	(1240)	(1351)	(1383)	(1373)	(1333)
Jackknife	98.6	98.1	97.7	97.5	97.0
	(1737)	(1419)	(1298)	(1163)	(1027)

[a]See Appendices A and B for description of interval estimators.

Stratified Replicated Systematic Sampling (STRSYS)

The advantages of stratification and replication in systematic sampling can be readily combined. The point and variance estimates obtained daily using RSYS can be added across days in a straightforward manner to obtain STRSYS estimators. The only requirement is independent randomization of sampling times on a daily basis.

The general form of the point estimate and sample variance for total fish passage using STRSYS over $j = 1, \ldots, D$ days can be written as

$$(18)\quad \hat{Y}_{\text{STRSYS}} = \sum_{j=1}^{D} \hat{Y}_{j,\text{RSYS}}$$

and variance estimate

$$(19)\quad \hat{\text{Var}}(\hat{Y}_{\text{STRSYS}}) = \sum_{j=1}^{D} \hat{\text{Var}}(\hat{Y}_{j,\text{RSYS}}) = \sum_{j=1}^{D} \left[\frac{(1 - f_j)}{l_j (l_j - 1)} \sum_{i=1}^{l_j} (\hat{Y}_{ij} - \hat{Y}_{j,\text{RSYS}})^2 \right]$$

where

$$\hat{Y}_{j,\text{RSYS}} = \sum_{i=1}^{l_j} \frac{\hat{Y}_{ij}}{l_j}$$

and where f_j = sampling fraction for the jth ($j = 1, \ldots, D$) (e.g., $In/60$) stratum, l_j = number of replicate SYS samples drawn in the jth stratum ($j = 1, \ldots, D$), and $\hat{Y}_{ij}$ = estimate of total fish passage based on the ith replicate SYS sample ($i = 1, \ldots, l_j$) in the jth stratum ($j = 1, \ldots, D$). Unbiased point and variance estimates are obtained using STRSYS.

Methods

Six multiday data sets were constructed from the original 15 d of continuous hydroacoustic monitoring data to evaluate the precision of the five alternative sampling designs. The individual data sets were constructed by grouping contiguous or near-contiguous days at a particular hydro-project together. Evaluation was based on comparison of sampling variances (i.e., $\text{Var}(\hat{Y})$) for the alternative sampling designs. For purposes of comparison, the level of sampling effort within an hour was the same for all designs. Sampling effort for SYS, STRS, and STSYS was specified by the product nl whereas the product lnl specified effort for RSYS and STRSYS. The true variances for each sampling design across the six alternative data sets were calculated using either exhaustive data sampling techniques or variance formula for finite sampling (Table 1). Monte Carlo simulation studies were used to evaluate confidence interval performance.

A total of 10 confidence interval estimation procedures using normality assumptions (Appendix A) and seven using bootstrap and jackknife methods (Appendix B) were investigated to find the most appropriate estimation techniques to use in conjunction with hydroacoustic surveys. The best confidence interval procedure was defined as that estimator with the properties of (1) attaining nominal coverage and (2) possessing minimal interval width. Since it might be expected that an interval estimate might not always exactly attain nominal coverage, an interval estimator should always equal or exceed nominal coverage to be valid. Excessive coverage, however, can result in unnecessarily wide interval estimates and perceived lower precision. This paper summarizes the results for the more predominant of the interval estimators (Appendices A and B). A complete review of confidence interval estimators in conjunction with finite sampling can be found in Skalski et al. (1989).

Results

Monte Carlo simulations were used to evaluate the five alternative sampling designs. Evaluations were based on the statistical properties of the estimators of total fish passage ($\hat{Y}$), sampling variances ($\text{Var}(\hat{Y})$), estimators of sampling variance ($\hat{\text{Var}}(\hat{Y})$), and average width and percent coverage of 95% confidence interval estimators. Performance was evaluated individually and relative to one another. Results are summarized first by individual sampling design and then discussed jointly.

TABLE 3. Percent relative error from 15 000 Monte Carlo simulations (i.e., 15 data sets × 1000 simulations/set) using SYS in estimating the sampling variance of the estimate of total fish passage. Percent relative error is expressed as 100% $(\overline{\text{Vâr}(\hat{Y})} - \text{Var}(\hat{Y}))/\text{Var}(\hat{Y})$ and averaged over the data sets.

Sampling effort (n/l)	Relative error			
	$\hat{V}_1$	$\hat{V}_2$	$\hat{V}_3$	$\hat{V}_4$
2/6	85.8	24.4	21.4	19.3
3/4	60.1	7.2	4.4	7.5
4/3	293.4	196.2	189.4	200.4
6/2	136.0	65.9	61.4	79.5
12/1	89.3	54.7	53.5	63.9

STRS

The statistical properties of STRS estimators are well known (Cochran 1977). The simulation results confirm these findings for hydroacoustic data and suggest that STRS has, on the average, the smallest sampling error. Specific properties of STRS are summarized as follows:

(1) Estimator of total passage (5) is unbiased (i.e., $E(\hat{Y}) = Y$).

(2) Variance estimator (7) is unbiased (i.e., $E[\text{Vâr}(\hat{Y}_{ST})] = \text{Var}(\hat{Y})$).

(3) On the average, STRS has the smallest sampling variance among designs compared (Table 1).

(4) Confidence interval estimators had nominal coverage (Table 2). Among alternative estimators, bootstrap and jackknife interval estimators had the worst performances (i.e., excessive width or far below nominal coverage) (Table 2). Among the normal theory confidence interval estimators, the expanded log interval estimator (Appendix A) had interval coverage closest to 95%.

(5) Sampling variance decreased predictably and monotonically with increased sampling effort (Table 1).

SYS

Our investigations suggest that use of this sampling design is based more on logical considerations than statistical performance. Specific properties of SYS are summarized as follows:

(1) Estimator of total passage (8) is unbiased (i.e., $E(\hat{Y}) = Y$).

(2) No consistent estimator for the variance (9) exists under all sampling conditions; in other words, variance estimates are biased (i.e., $E[\text{Vâr}(\hat{Y}_{SYS})] \neq \text{Var}(\hat{Y}_{SYS})$).

(3) On occasion, SYS can have a much smaller sampling variance than the other sampling designs; however, on the average, it is greater than STRS (Table 1).

(4) The four variance estimators investigated all overestimated the true variance, and in some circumstances overestimated the true variance by 100–300% (Table 3).

(5) Confidence interval coverage is somewhat erratic, fluctuating between 88 and 100% for 95% confidence intervals. Coverage is often close enough to nominal to be of practical value (Table 4).

(6) The tendency to overestimate the sampling variance of SYS (Table 3) results in the average confidence interval width being greater than or equal to that of STRS, effectively negating occasional improvements in sampling precision with SYS (i.e., comparison of Tables 2 and 4).

(7) Increasing sampling effort does *not* guarantee decreases in sampling variance (see Tables 1 and 5). There is no monotonic relationship between sampling effort and sampling variance (Table 5), making predictions of sampling precision with alternative levels of effort impossible.

RSYS

A solution to the lack of a consistent or reliable variance estimator in SYS is to physically replicate the SYS sample. In this manner, an empirical variance among estimates can be computed and subsequently used in interval estimation. Simulation results indicate that precision is lost using RSYS relative

TABLE 4. Average confidence interval coverage (%) for 95% confidence intervals and widths (parentheses) for SYS of 12 min/h using variance estimator $\hat{V}_4$. Data presented are averaged over the 15 data sets. Exhaustive sampling techniques resulted in 75 simulations total.

Interval estimator[a]	n/l				
	2/6	3/4	4/3	6/2	12/1
Z	92.0	88.0	97.3	89.3	93.3
	(1174)	(1079)	(1037)	(965)	(886)
log	92.0	88.0	97.3	90.7	94.7
	(1186)	(1087)	(1045)	(971)	(891)
Expanded log	92.0	89.3	97.3	92.0	94.7
	(1214)	(1107)	(1065)	(985)	(903)
Square root	92.0	89.3	97.3	89.3	93.3
	(1174)	(1079)	(1037)	(965)	(886)
Bootstrap	100.0	92.0	98.7	94.7	96.0
	(1516)	(1464)	(1364)	(1220)	(1006)
Nonparametric bootstrap	98.7	93.3	98.7	94.7	96.0
	(1620)	(1445)	(1363)	(1208)	(1066)
Bootstrap (RE)	100.0	97.3	100.0	98.7	100.0
	(2299)	(2049)	(1923)	(1737)	(1529)
Jackknife	100.0	93.3	98.7	94.7	97.3
	(1653)	(1464)	(1373)	(1238)	(1108)

[a]See Appendices A and B for description of interval estimators.

TABLE 5. Comparison of the sampling variance (i.e., $\mathrm{Var}(\hat{Y})$) of SYS for various levels of effort (i.e., nl) and within-hour allocations of that effort for data set IO515.

Effort (nl)	Allocation (n/l)	$\mathrm{Var}(\hat{Y})$
6	1/6	27 402
	2/3	13 047
	3/2	25 322
	6/1	17 404
12	1/12	5 044
	2/6	10 552
	3/4	17 674
	4/3	3 904
	6/2	11 238
	12/1	8 994
15	1/15	666
	3/5	1 227
	5/3	6 683
	15/1	1 919

to SYS for the gain in statistical validity and reliability. Specific properties of RSYS are summarized as follows:

(1) Estimator of total passage (16) is unbiased (i.e., $E(\hat{Y}_{\mathrm{RSYS}}) = Y$).

(2) Variance estimator (17) is unbiased (i.e., $E[\hat{\mathrm{Var}}(\hat{Y}_{\mathrm{RSYS}})] = \mathrm{Var}(\hat{Y}_{\mathrm{RSYS}})$).

(3) On the average, RSYS has the largest sampling variance among sampling designs compared (Table 1).

(4) Confidence interval estimators had nominal coverage (Table 6). Interval estimates based on log-transformed data had similar coverage rate but wider interval estimates than those confidence intervals based on untransformed data (Table 6). Hence, transformations of the estimates before calculation of confidence intervals in RSYS appear unnecessary.

(5) Confidence intervals based on RSYS had shortest width when numerous replicates with nominal sampling per hour were sampled (Table 6). The reason is that confidence intervals for RSYS are based on t-statistics where degrees of freedom depend on the number of replicates (i.e., $l - 1$) sampled.

(6) For equal sampling effort and ideal allocation of effort within RSYS, STRS had shorter interval estimates (Table 7).

STSYS

The well-known benefits for stratification in conjunction with random sampling (i.e., STRS) may also exist with SYS. However, stratification will not resolve the problem of a biased variance estimator. Specific properties of STSYS are summarized as follows:

(1) Estimator of total passage (14) is unbiased (i.e., $E(\hat{Y}) = Y$).

(2) Variance estimators are biased (i.e., $E[\hat{\mathrm{Var}}(\hat{Y}) \neq \mathrm{Var}(\hat{Y})]$.

(3) Stratification in conjunction with systematic sampling

TABLE 6. Summary of the performance of RSYS for alternative allocations of effort for l/n = 12 min for 15 d of continuous hydroacoustic monitoring. Summary includes average sampling variance, interval coverages, and average 95% confidence interval width for untransformed and ln-transformed estimates of total fish passage.

Sampling effort				Untransformed		ln-transformed	
l	n	I(min)	Average variance	% coverage	Average width	% coverage	Average width
2	1	6	366 232	96.0	5255	96.2	286 913
3	1	4	283 662	95.2	1697	95.1	1 782
4	1	3	239 571	95.2	1739	95.7	1 855
2	2	3	99 289	94.8	6552	95.0	272 339
6	1	2	198 945	96.6	1372	97.0	1 431
3	2	2	95 765	93.9	2175	94.2	2 589
2	3	2	170 574	95.3	5800	95.3	2 436 921
12	1	1	129 339	96.4	1361	97.2	1 414
6	2	1	80 424	96.8	1965	97.1	2 174
4	3	1	122 203	95.6	2135	95.8	2 603
3	4	1	60 022	96.0	2903	96.2	7 984
2	6	1	84 488	93.0	8320	93.6	33 443

TABLE 7. Comparison of the average variance, percent coverage, and average 95% confidence interval width for STRS and RSYS for the 15 d of 24-h continuous hydroacoustic monitoring for equal sampling effort under different allocations of effort.

STRS				RSYS			
n/l	Average variance	% coverage	Average width	$l/n/l$	Average variance	% coverage	Average width
2/6	113 090	93.2	1158	2/1/6	366 232	96.0	5255
3/4	93 724	94.1	1075	3/1/4	283 662	95.2	1697
4/3	84 871	94.1	1032	4/1/3	239 571	95.2	1739
6/2	72 087	94.8	961	6/1/2	198 945	96.6	1372
12/1	58 749	95.0	878	12/1/1	129 339	96.4	1361

TABLE 8. Confidence interval coverage for 95% confidence intervals and widths (parentheses) for SYS, STSYS, and STRSYS sampling schemes for various allocations of effort using multiday continuous hydroacoustic monitoring data.

Data set	*n/l*	*l/n/l*	SYS[a,b]	STSYS[a]	STRSYS[c]
1					
RO521	2/6	2/1/6	80	93.4	94.4
			(2389)	(2377)	(3161)
RO522	3/4	3/1/4	80	87.2	95.3
			(2221)	(2226)	(2500)
RO524	4/3	4/1/3	100	99.4	95.1
			(2184)	(2187)	(2465)
RO526	6/2	6/1/2	60	77.9	95.1
			(2009)	(2014)	(2280)
	12/1	12/1/1	80	93.1	95.1
			(1852)	(1850)	(1878)
2					
R513	2/6	2/1/6	100	98.4	92.6
			(4476)	(4453)	(10553)
R514	3/4	3/1/4	40	77.1	94.1
			(3872)	(3874)	(7836)
R520	4/3	4/1/3	100	99.2	94.2
			(3669)	(3670)	(6766)
R521	6/2	6/1/2	100	98.6	94.1
			(3367)	(3367)	(5733)
	12/1	12/1/1	100	98.1	94.9
			(3003)	(3003)	(4422)
3					
RO521	2/6	2/1/6	80	96.2	91.2
			(3403)	(3396)	(4998)
RO522	3/4	3/1/4	80	98.6	94.4
			(3187)	(3188)	(4062)
R513	4/3	4/1/3	100	99.8	94.5
			(3052)	(3055)	(3658)
R514	6/2	6/1/2	100	99.2	94.9
			(2833)	(2833)	(3153)
	12/1	12/1/1	80	96.2	94.9
			(2607)	(2608)	(2774)
4					
RO521	2/6	2/1/6	80	98.4	92.3
			(3789)	(3785)	(9731)
RO522	3/4	3/1/4	20	63.7	93.0
			(3217)	(3217)	(7201)
R520	4/3	4/1/3	100	99.4	93.0
			(3006)	(3008)	(6085)
R521	6/2	6/1/2	80	90.6	93.1
			(2782)	(2785)	(5278)
	12/1	12/1/1	100	96.6	94.4
			(2441)	(2441)	(3915)
5					
RO524	2/6	2/1/6	60	94.6	91.8
			(3336)	(3322)	(5097)
RO526	3/4	3/1/4	80	98.1	94.0
			(3102)	(3102)	(3899)
R513	4/3	4/1/3	80	99.7	94.6
			(3024)	(3032)	(3764)
R514	6/2	6/1/2	80	95.4	94.9
			(2762)	(2762)	(3183)
	12/1	12/1/1	100	96.5	94.9
			(2547)	(2545)	(2781)
6					
RO524	2/6	2/1/6	100	98.4	92.9
			(3747)	(3727)	(10163)
RO526	3/4	3/1/4	60	62.7	92.4
			(3130)	(3135)	(7195)

TABLE 8. (*Concluded*)

Data set	*n/l*	*l/n/l*	SYS[a,b]	STSYS[a]	STRSYS[c]
R520	4/3	4/1/3	100	100.0	93.3
			(2987)	(2987)	(6221)
R521	6/2	6/1/2	60	82.1	93.3
			(2713)	(2715)	(5330)
	12/1	12/1/1	100	98.2	94.5
			(2324)	(2374)	(3931)

[a]Confidence interval calculation based on expanded log (Appendix A).
[b]Confidence coverage based on a result of five exhaustive sample alternatives.
[c]Confidence interval calculation based on expanded log using a *t*-statistic with $(l - 1)D$ degrees of freedom rather than a *Z*-statistic.

(STSYS) can reduce the sampling variance over SYS and produces results near those of STRS (Table 1).

(4) Confidence interval estimates for STSYS have the same average interval width as those of SYS (Table 8) despite reduced sampling variance (Table 1). The reason for similar interval widths is the similar use of approximate variance estimator $\hat{V}_4$.

(5) Confidence interval coverage is generally near nominal for 95% interval estimates (Table 8).

STRSYS

The benefits of stratification can also be realized in conjunction with the statistical advantages of RSYS. Specific properties of STRSYS are summarized as follows:

(1) Estimator of total passage (18) is unbiased (i.e., $E(\hat{Y}_{STRSYS}) = Y$).

(2) Variance estimator (19) is unbiased (i.e., $E[\hat{\text{Var}}(\hat{Y}_{STRSYS})] = \text{Var}(\hat{Y}_{STRSYS})$).

(3) Stratification provides a moderate reduction in sampling variance over RSYS (Table 1).

(4) Confidence intervals have nominal coverage with widths generally in excess of SYS or STSYS except when numerous replicate samples are collected (Table 8).

Comparison of the Alternative Sampling Designs

All five sampling designs provide unbiased point estimates (i.e., $E(\hat{Y}) = Y$); however, only STRS, RSYS, and STRSYS provide unbiased variance estimates (i.e., $E[\hat{\text{Var}}(\hat{Y})] = \text{Var}(\hat{Y})$) for subsequent interval estimation and expression of sampling precision.

Ranking the variances for the five sampling designs from smallest to largest (Table 1) produced the ordering (STRS $\approx$ STSYS) < (SYS $\approx$ STRSYS) < RSYS. STRS sampling typically had the smallest sampling variance, followed closely by STSYS. Classical SYS and STRSYS had similar rank orders but generally larger variances than either STRS or STSYS. Finally, RSYS ranked the worst with the largest variance.

Inspection of $\text{Var}(\hat{Y})$ for STRS shows a trend of decreasing variance as the number of samples per hour (n) increases and interval width (l) decreases for the same overall level of sampling effort nl. For both SYS and STSYS, no consistent pattern in $\text{Var}(\hat{Y})$ exists as sample allocation changes; with erratic jumps in variance possible (see Data Set 5, Table 1), making selection of proper sampling effort difficult, at best. The erratic behavior of $\text{Var}(\hat{Y})$ using SYS extends to changes in overall sampling effort, as well as shifts in the distribution of that effort (Table 5). In SYS, there is little or no expectation that increasing sampling effort will necessarily improve the precision of the monitoring study (Table 5). This paradox makes implementation of SYS to achieve a prescribed level of precision nearly impossible. In the case of RSYS and STRSYS, numerous replicate SYS samples with short duration (i.e., $l = 1$ min) and no within-hour replicate (i.e., $n = 1$) produced the smallest sampling variance. Skalski et al. (1989) showed similar results for sampling precision for levels of effort other than that presented here.

The four alternative variance estimators of total fish passage for SYS (equations (10), (11), (12), and (13)) perform poorly in providing reliable variance estimates (Table 3). Averaging across the sampling results for the 15 d of continuous hydroacoustic monitoring data, all four estimators show a trend to overestimate the true variance (Table 3). Consequently, the variance estimators investigated provide valid but conservative estimates of sampling variance. Generally, we recommend using the stratified random sample variance (i.e., $\hat{V}_4$) in conjunction with SYS because of its familiarity, computational ease, and performance. The general performance of the variance estimators for SYS to either over- or underestimate the true variance is unrelated to sampling effort (Table 3) of the survey.

In general, STRS had the shortest confidence interval widths while maintaining nominal 95% coverage. Problems in providing consistent variance estimates for SYS carried over to interval estimation. Although SYS often had smaller sampling variance than STRS, widths of confidence intervals did not reflect this sampling precision. For the same level of hydroacoustic sampling effort, 95% confidence intervals using SYS had much wider interval widths than STRS (comparison, Tables 4 and 5) using comparable interval estimators. The tendency for variance estimator $\hat{V}_4$ to exceed the magnitude of the true variance may in part explain the wider interval widths but does little to explain the less than nominal coverage for normal theory estimators (Table 5). Overall, bootstrap and jackknife estimators provided nominal or greater coverage than 95% at the expense of the widest interval estimators. Confidence interval estimators with SYS, although somewhat erratic in behavior, typically provide valid but conservative 95% interval estimates. We recommend interval estimates for SYS be based on variance estimator $\hat{V}_4$ using the expanded log algorithm (Table 4) and not on the computationally intensive bootstrap or jackknife methods. Similar recommendations apply to the other sampling designs.

Comparison of confidence interval estimates for STRS and RSYS clearly shows interval widths for RSYS being much wider than interval widths for STRS for the same sampling effort (Table 7). Hence, although RSYS can rectify some of

the shortcomings of ordinary SYS, price of the improvement is a loss of precision over STRS methods and SYS itself. Comparison of SYS, STSYS, and STRSYS indicates that stratification by day, in conjunction with SYS, has little effect on precision, while STRSYS approaches the precision of other methods, only when numerous replicate samples (Table 8) are employed.

Discussion

Currently, SYS is the standard sampling design for fixed-location hydroacoustic monitoring of salmon smolt outmigration in the Columbia River. Monte Carlo simulation studies have shown that valid but often conservative confidence interval estimates of total fish passage can be calculated from SYS monitoring data by treating the data as if they had been generated using STRS. The recommended variance estimator $\hat{V}_4$ for use with SYS will typically overestimate the true variance (also see Wolter 1984 for similar results) and consequently mask the actual precision of the study. An unavoidable problem with SYS is that precision of the surveys does not change monotonically with increasing sampling effort. One cannot predict the anticipated precision of SYS based solely on sampling effort. The consequence is that the performance of the monitoring study is held hostage to the vagaries of the outmigration process being sampled. An important improvement over ordinary SYS is the use of replication (i.e., RSYS). No increase in field costs are anticipated, yet the resulting survey data provide reliable variance estimates and extremely well-behaved confidence interval estimates. For RSYS and STRSYS to exhibit reasonable precision, however, the sampling design must consist of numerous replicates (i.e., $l > 10$), each of which samples fish passage for a short duration each hour (i.e., 1 min).

Of all the sampling designs investigated, STRS holds the greatest promise for fixed-location hydroacoustic monitoring. STRS yields unbiased point and variance estimates, nominal confidence interval behavior, shortest interval widths, and stable variance relationships that can be used to project sampling precision under alternative allocations of effort. These attributes recommend STRS over existing SYS and its modifications investigated in this paper. The limiting factor in applying STRS to existing monitoring studies is that new electronic hardware must be developed permitting the stochastic switching of transducers during monitoring. However, importance of statistically defensible and precise hydroacoustic estimates in salmon management on the Columbia River will hasten further improvements in sampling theory and methods, including implementation of STRS.

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Appendix A: Parameter Interval Estimates

Estimators for STRS, SYS, and STSYS

Z-Interval

In the case of a 95% confidence interval, $Z_{1-\alpha/2} = 1.96$ and so

$$\mathrm{CI}(\hat{Y} - 1.96\sqrt{\hat{\mathrm{Var}}(\hat{Y})}, \hat{Y} + 1.96\sqrt{\hat{\mathrm{Var}}(\hat{Y})}) = 0.95 .$$

For the remainder of the text, we will consider only 95% confidence interval estimates.

Lognormal

Assuming

$$\log(\hat{Y}) \sim N\left(\log(Y), \frac{\mathrm{Var}(\hat{Y})}{Y^2}\right),$$

then an approximate 95% confidence interval for $\log(Y)$ is

$$\mathrm{CI}\left(\hat{Y}\exp\left\{-1.96\sqrt{\frac{\hat{\mathrm{Var}}(\hat{Y})}{\hat{Y}^2}}\right\},\right.$$

$$\left.\hat{Y}\exp\left\{1.96\sqrt{\frac{\hat{\mathrm{Var}}(\hat{Y})}{\hat{Y}^2}}\right\}\right) = 0.95 .$$

Expanded lognormal

Assuming

$$\log(\hat{Y}) \sim N\left(\log(Y), \mathrm{Var}(\hat{Y})\left(\frac{1}{Y^2} + \frac{3\mathrm{Var}(\hat{Y})}{Y^4}\right)\right),$$

then an approximate 95% confidence interval for Y is

$$\text{CI}\left(\hat{Y}\exp\left\{-1.96\sqrt{\hat{\text{Var}}(\hat{Y})\left(\frac{1}{\hat{Y}^2}+\frac{3\hat{\text{Var}}(\hat{Y})}{\hat{Y}^4}\right)}\right\},\right.$$

$$\left.\hat{Y}\exp\left\{1.96\sqrt{\hat{\text{Var}}(\hat{Y})\left(\frac{1}{\hat{Y}^2}+\frac{3\hat{\text{Var}}(\hat{Y})}{\hat{Y}^4}\right)}\right\}\right) = 0.95.$$

Square root

Assuming

$$\sqrt{\hat{Y}} \sim N\left(\sqrt{Y}, \frac{\hat{\text{Var}}(\hat{Y})}{4Y}\right),$$

then an approximate 95% confidence interval for Y is

$$\text{CI}\left(\left(\sqrt{\hat{Y}} - 1.96\sqrt{\frac{\hat{\text{Var}}(\hat{Y})}{4\hat{Y}}}\right)^2,\right.$$

$$\left.\left(\sqrt{\hat{Y}} + 1.96\sqrt{\frac{\hat{\text{Var}}(\hat{Y})}{4\hat{Y}}}\right)^2\right) = 0.95.$$

Estimators for RSYS and STRSYS

Because the estimate of total fish passage in RSYS is based on a mean of l replicate samples, an empirical variance can be calculated based on $l - 1$ degrees of freedom. Consequently, assuming approximate normality of the average estimate using the Central Limit Theorem, confidence interval estimates for RSYS can be based on the t-distribution with $l - 1$ degrees of freedom. A $(1 - \alpha)100\%$ confidence interval is computed as

$$\hat{Y}_{\text{RSYS}} \pm t_{1-\alpha/2,\, l-1}\sqrt{\hat{\text{Var}}(\hat{Y}_{\text{RSYS}})}.$$

An alternative interval estimator based on a ln-transformation can be written as

$$\hat{Y}_{\text{RSYS}}\, e^{\pm t_{1-\alpha/2,l-1}\sqrt{\text{Var}(\hat{Y})\left(\frac{1}{\hat{Y}^2}+\frac{3\text{Var}(\hat{Y})}{\hat{Y}^4}\right)}}.$$

For STRSYS, the confidence interval estimate is based on a t-statistic with $(l - 1)D$ degrees of freedom. Throughout this paper, confidence intervals for RSYS and STRSYS were based on the expanded ln-transformation.

Appendix B: Nonparametric Interval Estimators

Standard Bootstrap

From hydroacoustic monitoring, n sample observations are collected. These may be the n elements sampled each hour as in STRS or the n elements sampled as in SYS.

Let $\underset{\sim}{y} = (y_1, \ldots, y_n)$ be a sample.

Let $\underset{\sim}{y}^*_i = (y^*_{1i}, \ldots, y^*_{ni})$ be a bootstrap sample i obtained from $\underset{\sim}{y}$ where each y^*_x is randomly drawn from $\underset{\sim}{y}$ with replacement.

From $\underset{\sim}{y}^*_i$, we calculate an estimate $\hat{Y}^*_i$ of fish passage as we would have from $\underset{\sim}{y}$. We repeat this process B times. For each sample, we have B such estimates $\{\hat{Y}^*_i, i = 1, \ldots, B\}$.

The bootstrap estimate of variance is the empirical variance of the $\{\hat{Y}^*_i\}$, i.e.,

$$\hat{\text{Var}}^*(\hat{Y}^*) = \frac{\sum_{i=1}^{B}(\hat{Y}^*_i - \bar{Y}^*)^2}{B-1}$$

where

$$\bar{Y}^* = \frac{1}{B}\sum_{i=1}^{B}\hat{Y}^*_i.$$

In SYS, $\hat{\text{Var}}^*(\hat{Y}^*)$ is the estimate of the sampling variance. In STRS, $\hat{\text{Var}}^*(\hat{Y}^*)$ is the hourly estimate of variance. Because of the assumptions of independence, these hourly estimates in STRS are calculated for each hour and then summed for the daily estimate. In our simulation studies, we set $B = 200$.

Assuming

$$\hat{Y}^* \sim N(Y, \text{Var}^*(\hat{Y}^*)),$$

an approximate 95% confidence interval for Y is calculated using a Z-interval.

Nonparametric Bootstrap

This procedure is nonparametric. We calculated the 200 bootstrap estimates ($\hat{Y}^*_i$, $i = 1, \ldots, 200$). After ordering these, we calculated a 95% confidence interval whose end points are

$$(\hat{Y}^*_{(5)}, \hat{Y}^*_{(195)})$$

where $\hat{Y}^*_{(i)}$ is the ith-order bootstrap estimate.

Bootstrap (Random Error)

By adding a random error term to the original observations, a more continuous distribution can be generated with anticipated improved confidence interval performance.

Let $\underset{\sim}{y} = (y_1, \ldots, y_n)$ be the original sample observations as above.

Let $\underset{\sim}{y}'_i = (y'_{1i}, \ldots, y'_{ni})$ be a modified bootstrap sample i where each y'_x is computed as shown below.

Let $\underset{\sim}{e}'_i = (e'_{1i}, \ldots, e'_{ni})$ where

$$e_x = y^*_x - \bar{y}^*_i$$

and

$$\bar{y}^*_i = \frac{1}{n}\sum_{j=1}^{n} y^*_{ij}.$$

Let $y'_{ij} = y^*_x + e'_x$ where y^*_x is randomly drawn from $\underset{\sim}{y}$ with replacement and e_x is randomly drawn from $\underset{\sim}{e}'_i$ with replacement.

Adding the random error component to the original bootstrap data to create the modified bootstrap data (denoted by RE for random error) increases the bootstrap population by a factor of n. It does this in an unbiased manner, since the errors are all calculated from the mean.

As in the unmodified case, we calculate an estimate $\hat{Y}'_i$ of fish passage from $\underset{\sim}{y}'_i$. For each sample, we repeat this process B times to obtain $\{\hat{Y}'_i, i = 1, \ldots, B\}$ from which we calculate the empirical variance (i.e., $\hat{\text{Var}}^*(\hat{Y}')$) as our variance estimate. Again, we set $B = 200$.

Assuming

$$\hat{Y}^* \sim N(Y, \text{Var}^*(\hat{Y}^*)),$$

an approximate 95% confidence interval for Y is calculated using a Z-interval.

Jackknife

Because SYS does not provide a consistent variance estimate, the jackknife method provides an opportunity to rectify the variance estimate to improve confidence coverage.

Let $\underset{\sim}{y} = (y_1, \ldots, y_n)$ be the sample of original observations.

Let $\underset{\sim}{y}_i = (y_1, \ldots, y_{i-1}, y_{i+1}, \ldots, y_n)$ be $\underset{\sim}{y}$ with y_i deleted.

From this sample, calculate n estimates of fish passage $\{\hat{Y}_i, i = 1, \ldots, n\}$ where

$\hat{Y}_i$ = fish passage estimated from $\underset{\sim}{y}_i$.

The jackknifed estimate of variance is

$$\hat{\text{Var}}(\hat{Y}) = \frac{n-1}{n} \sum_{i=1}^{n} (\hat{Y}_i - \hat{Y}_\cdot)^2$$

where

$$\hat{Y}_\cdot = \frac{1}{n} \sum_{i=1}^{n} \hat{Y}_i .$$

Assuming

$$\hat{Y} \sim N(Y, \text{Var}(\hat{Y})),$$

we approximate $\text{Var}(\hat{Y})$ by $\hat{\text{Var}}(\hat{Y})$. An approximate 95% confidence interval for Y is computed using a Z-interval.

For more details on the jackknife and bootstrap procedures, see Efron (1982).

Use of Biochemical Genetic Markers to Discriminate between Adductor Muscles of the Sea Scallop (*Placopecten magellanicus*) and the Iceland Scallop (*Chlamys islandica*)

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Three biochemical techniques were applied to adductor muscles of *Placopecten magellanicus* and *Chlamys islandica*, two commercially important scallops, to search for species-specific genetic markers. Allozyme electrophoresis identified two enzyme systems, mannose phosphate isomerase and glutamic oxaloacetate transaminase, which appear to be diagnostic. Sequencing of the 18S rRNA gene in each species showed 15 nucleotide differences over 1815 base pairs. Digestion of the gene with the restriction enzyme XHO I released two fragments in *Placopecten* and three in *Chlamys*. All three techniques could be developed for management purposes as part of an enforcement programme to identify illegally caught scallops.

Nous avons appliqué trois techniques biochimiques aux muscles adducteurs de *Placopecten magellanicus* et *Chlamys islandica*, deux espèces de pétoncles commercialement importantes, pour rechercher des marqueurs génétiques particuliers à chaque espèce. L'électrophorèse des allozymes a permis d'identifier deux systèmes enzymatiques, la mannose-phosphate-isomérase et la glutamate-oxaloacétate-transaminase, qui semblent avoir un pouvoir diagnostique. Le séquençage du gène 18S de l'ARNr chez chaque espèce a révélé 15 différences dans les nucléotides sur 1 815 paires de bases. La digestion du gène par l'enzyme de restriction XHO I a libéré deux fragments chez *Placopecten* et trois chez *Chlamys*. Il serait possible de développer ces trois techniques à des fins de gestion dans le cadre d'un programme d'application des règlements visant l'identification des pétoncles capturés illégalement.

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There are two scallop species fished commercially in Atlantic Canada. The sea scallop, *Placopecten magellanicus* (Gmelin, 1791), is the larger and more abundant of the two. Restricted to the northwest Atlantic Ocean, it constitutes the single most abundant and valuable naturally occurring scallop species in the world. The Iceland scallop, *Chlamys islandica* (Müller, 1776), is a circumpolar species (northern boreal or subarctic transition zone) and in Canada is fished sporodically. It is considered to be an underutilized species at present.

One of the management tools in the sea scallop fishery is that of size regulation. As most of the processing is carried out at sea, with only the adductor muscles (meats) commonly landed, the size regulations are based on meat counts. For most of Atlantic Canada the meat count regulation is set at 33 meats per 500 g. This has proven to be an effective management tool for the *Placopecten* fishery, but effective management of the *Chlamys* fishery requires a different (higher) meat count, as it is a smaller scallop. Different meat counts for the two fisheries would be difficult to enforce because it is impossible to separate the meats of the two species visually. Specific identification requires the presence of the shell. Large meats can be positively identified as *Placopecten* but small meats could be either species. A *Chlamys* fishery with a high meat count could land large numbers of juvenile *Placopecten*. Development of the *Chlamys* fishery is desirable, especially on the Newfoundland Banks where it is abundant but coexists with *Placopecten*. However, the use of species-specific size regulations, which are needed for effective management, depend upon an ability to positively identify the species of scallop from which the adductor muscle is drawn.

Allozyme frequencies have been used previously to distinguish genera of scallops (Beaumont 1991) and species of oys-

ters (Buroker et al. 1979; Buroker 1982) and mussels (McDonald and Koehn 1988). Such a tool, if proven diagnostic in separating *Placopecten* from *Chlamys*, would be invaluable to fishery enforcement officers in detecting mixed catches. Allozyme electrophoresis is a relatively inexpensive protocol, thus rendering such an approach feasible for large-scale sampling. However, in most studies (e.g., Beaumont 1991), tissue has been immediately frozen upon removal from the animal, or taken fresh, to avoid enzyme degradation. If such sampling is vital to the success of the results, allozyme electrophoresis would not be useful in sampling a fisherman's catch which may have been shucked (adductor muscle removed from the whole animal) up to 12 d before landing (maximum time once enforced by regulation, but now market controlled).

In order for allozyme electrophoresis to be used as a diagnostic tool, homologous loci must have diverged to the point that all individuals of one species have different enzyme forms than the individuals of the other species. Identification of species-specific alleles is complicated by the extent of intraspecific variation in allozyme frequencies which can have both a geographic and a within-population element. In *P. magellanicus*, genetic similarity would appear to be high between different populations in different parts of the species' range (Volckaert and Zouros 1989; Beaumont and Zouros 1991; Volckaert et al. 1991). Such data are not available for *C. islandica* on the east coast of North America; however, Fevolden (1989) has documented polymorphism in three enzyme systems for this species from the northeastern Atlantic Ocean. In one of these systems (glucose phosphate isomerase) the pattern of variation was strongly linked to geographic location. Similarly, studies on two congeners, *C. opercularis* and *C. varis*, have shown intraspecific variability in some loci (*C. opercularis*: Beaumont and Gruffydd 1975; Mathers 1975; Beaumont 1982; *C. varis*: Gosling and Burnell 1988). Thus, it would be essential to sample numerous populations of *C. islandica* before intergeneric diagnostic loci could be established.

An alternative approach to allozyme surveys is DNA analysis. DNA is much more stable than enzymes, and DNA analysis is a more direct method of determining species identity. However, the costs involved are prohibitive when large numbers of samples must be analyzed. DNA sequencing has been proposed for use in fisheries enforcement to identify illegal catches of tuna (Bartlett and Davidson 1991). Direct sequencing of the mitochondrial genes coding for cytochrome *b* was useful in identifying four species of tuna (*Thunnus* sp.). In this fishery, poachers destroy the diagnostic morphological features of the fish to evade detection. DNA sequencing was effective in separating the species from meat samples whereas protein electrophoresis was not.

In scallops, the gene sequence coding for the small subunit of ribosomal RNA (18S rDNA) has been published for *P. magellanicus* (Rice 1990: EMBL accession No. X53899). This gene has been sequenced in over 100 species (Dams et al. 1990) and has proven useful in separating genera (Sogin et al. 1989) and as a phylogenetic character (Cedergren et al. 1988; Field et al. 1988; Sogin et al. 1989; Bird et al. 1992). Intraspecific variation in this gene is not expected to be extensive, and in the only study to test this hypothesis, Bird et al. (1992) found only insignificant variation (within the error of the method) within species of red algae.

An alternative approach to sequencing the 18S-rRNA gene is to examine restriction fragment polymorphisms. If the gene sequences differ, they are likely to have different recognition sites for restriction enzymes. The DNA can then be digested with the appropriate enzyme and the digest fragment sizes compared by electrophoresis on an agarose gel. This approach is not as expensive as sequencing and is amenable to larger sample sizes.

We have used allozyme electrophoresis, DNA sequencing, and DNA restriction fragment length polymorphisms to identify diagnostic techniques for discriminating between adductor muscles of *P. magellanicus* and *C. islandica*.

Materials and Methods

Allozyme Electrophoresis

Samples of *P. magellanicus* and *C. islandica* were collected from St. Pierre Bank (46°28′N; 57°01′W) from 41–44 m in January 1988. Scallops were transported live in a running seawater system aboard the research vessel *Gadus Atlantica* to the Northwest Atlantic Fisheries Centre in St. John's, Newfoundland (18 h), and subsequently flown to the Biology Department, Dalhousie University, Halifax, Nova Scotia (2 h).

Portions of the adductor muscles were removed and samples from the same animals were treated in each of three ways (1) immediate storage at −80°C, (2) on ice for 12 d prior to storage at −80°C, and (3) used fresh. To determine if cross contamination of tissue by the enzymes from the two species could occur under normal storage conditions onboard ship, samples of tissue from the two species were placed in direct physical contact with each other and stored on ice for 12 d prior to analysis. The species were then separated after storage. For each species, 21 adductor tissues were scored fresh, 15 after immediate freezing, 15 after being on ice prior to freezing, and 24 after being in an intermixed collection on ice for 12 d and then processed. Portions (0.2 g) of fresh or thawed tissue were placed in tubes containing an equal volume of ice-cold water, homogenized, and centrifuged at 15 000 *g* for 10 min at 4°C. Supernatants were analyzed by starch gel electrophoresis in a 0.02 M Tris–citrate buffer system (pH 8.0) run for 9 h at 90 mA at 4°C. Gels were sliced into 3-mm-thick slabs and stained for three enzymes: phosphoglucomutase (PGM: EC 5.4.2.2), mannose phosphate isomerase (MPI: EC 5.3.1.8), and glutamate oxaloacetate transaminase (GOT: EC 2.6.1.1) as in Rice and Crowden (1987). These enzymes were chosen for their staining properties and stability in *Placopecten* (Foltz and Zouros 1984).

DNA Sequencing

The adductor muscles were removed from samples of *Placopecten* and *Chlamys* collected from the Bay of Fundy using the research vessel the *J.L. Hart* and frozen at −80°C onboard ship. High molecular weight DNA was extracted readily from the tissue following the method of Rice and Bird (1990). DNA was purified on cesium chloride gradients, although this step was found to be unnecessary, and the 18S rRNA genes were amplified by the polymerase chain reaction (PCR) protocol with TAQ polymerase (Bio-Can Scientific, Mississauga, Ontario). Synthetic oligonucleotides corresponding to evolutionary conserved regions of the gene containing the sequences 5′-dCAACCTGGTTGATCCTGCCAGT-3′ and 5′-dCTGATCCTTCTGCAGGTTCACCTAC-3′ and attached to restriction endonuclease sites (Bird et al. 1990; Sogin 1990) were used as amplification primers. A Perkin-Elmer (Irvine,

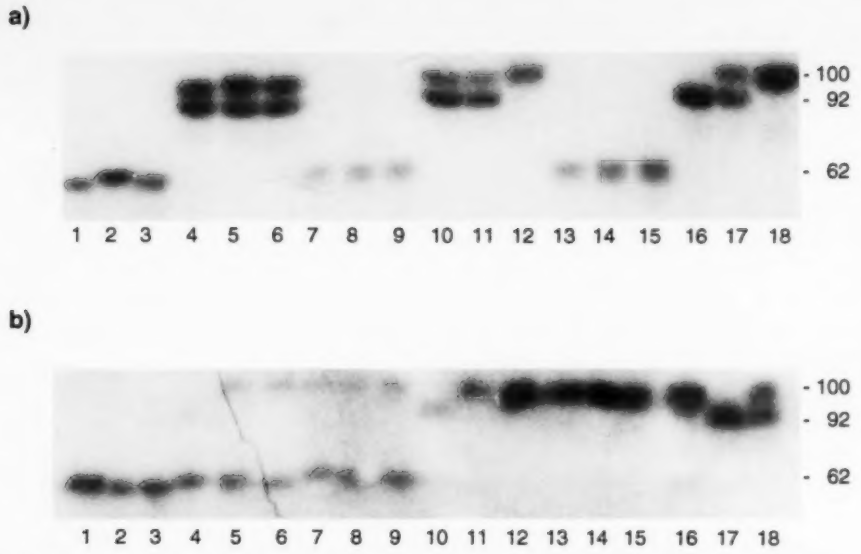

FIG. 1. Starch gel stained for mannose phosphate isomerase (MPI). (a) Scallops stored separately: lanes 1–3, *C. islandica* fresh tissue; lanes 4–6, *P. magellanicus* fresh tissue; lanes 7–9, *C. islandica* tissue kept on ice; lanes 10–12, *P. magellanicus* tissue kept on ice; lanes 13–15, *C. islandica* frozen at −80°C; lanes 16–18, *P. magellanicus* tissue frozen at −80°C. (b) Scallops stored together: lanes 1–3, *C. islandica* fresh tissue; lanes 4–9, *C. islandica* mixed storage; lanes 10–12, *P. magellanicus* fresh tissue; lanes 13–18, *P. magellanicus* mixed storage.

Calif.) DNA thermal cycler was used to cycle the temperature of the reaction mixtures. Amplifications were carried out with the following regime: denature for 5 min at 94°C, 30 cycles of annealing for 2 min at 37°C, extension for 3 min at 72°C and denaturing for 1 min at 94°C, followed by one annealing phase for 2 min at 37°C and one extension phase for 5 min at 72°C.

Amplified DNA was restricted with various endonucleases to screen for internal sites. The amplified DNA was digested with enzymes SAL I and BAM HI, which do not cut internally, and ligated into similarly restricted plasmid vector pUC18. The recombinant plasmids were used to transform (Hanahan 1986) competent *Escherichia coli* TB1 cells, and recombinant colonies were screened for inserts of the correct size. Purified plasmid DNA (Garger et al. 1983) was subjected to dideoxy sequencing (Sanger et al. 1977) according to the USB Sequenase version 2.0 protocol. Sequencing primers were in Bird et al. (1992). dITP was substituted for dGTP in the sequencing protocol as required in order to resolve secondary-structure compressions.

The DNA was read in both directions with complete overlap. The 18S rDNA sequences were assembled for each species using ESEE version 1.09d (E. Cabot, Department of Biology, University of Rochester, Rochester, NY 14627, 1990) on an IBM computer. Nucleotide differences were documented.

DNA Restriction Fragment Polymorphisms

The sequences were analyzed further for restriction sites using DNA STRIDER version 1.0 (Commissariat à l'Energie Atomique, France). DNA fragment sizes were screened for usefulness as genetic markers. XHO I digests the gene into fragments which can readily be detected on minigels. The plasmid DNA from each species was then restricted with XHO I and also with the enzymes SAL I and BAM HI (to release the insert) and electrophoretically fractionated in 0.7% agarose gels with TBE buffer (Maniatis et al. 1982) for 2 h at 50 V. Gels were stained with ethidium bromide to determine whether different DNA fragment sizes could be detected.

Results

Allozyme Electrophoresis

Mannose phosphate isomerase (MPI)

The allozyme bands are stronger staining and migrate faster in *Placopecten* than in *Chlamys* (Fig. 1a). In *Placopecten*, three of the individuals were homozygous whereas all others were heterozygous. The commoner band in this species was given the mobility index of 100 and the other mobilities were correspondingly assigned the values of 92, 73, and 62. The latter two are specific to *Chlamys*. Within *Chlamys*, the band with mobility of 62 was more common than the one of 73. Among the *Chlamys* individuals, most were homozygous for allele 62. There was no effect of pretreatment on allozyme appearance. Fresh, chilled, and frozen tissue all produced the same results (Fig. 1a). Intraspecific polymorphism does not interfere with the diagnosis of one species from the other. When the meats from each species were held together for 12 d prior to analysis, some transfer of the enzymes from one tissue to the other was observed (Fig. 1b). All banding patterns are as expected with the exception of the *Chlamys* held under mixed storage conditions. It would appear that when the meats are in direct contact with each other, there is a transfer of enzyme from one tissue to the other. This appears to be of more practical importance in the *Chlamys* meats, as any transfer in the reverse direction is not detectable on the gels. This may be because the enzyme concentration is higher in *Placopecten* than in *Chlamys*. The transfer does not affect the diagnostic power of the assay

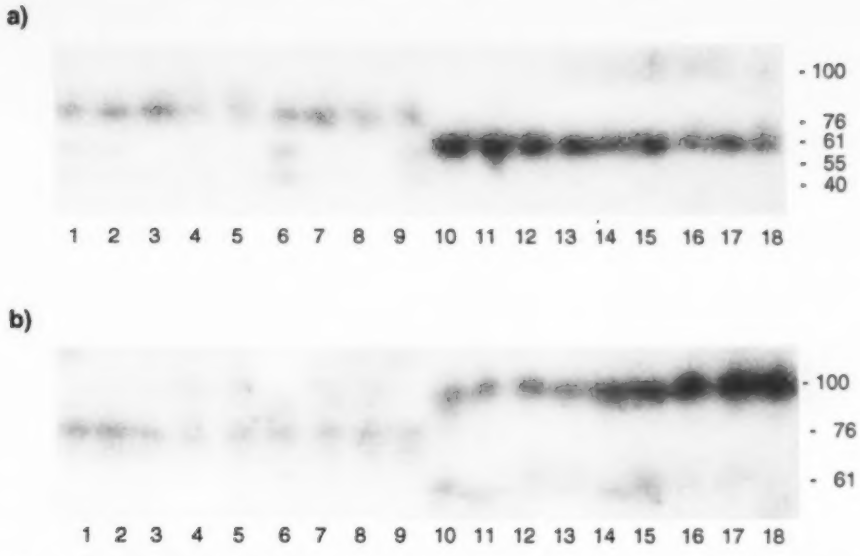

FIG. 2. Starch gel stained for glutamic oxaloacetate transaminase (GOT). (a) Scallops stored separately: lanes 1–3, *C. islandica* fresh tissue; lanes 4–6, *C. islandica* tissue kept on ice; lanes 7–9, *C. islandica* frozen at −80°C; lanes 10–12, *P. magellanicus* fresh tissue; lanes 13–15, *P. magellanicus* tissue kept on ice; lanes 16–18, *P. magellanicus* tissue frozen at −80°C. (b) Scallops stored together: lanes 1–3, *C. islandica* fresh tissue; lanes 4–9, *C. islandica* mixed storage; lanes 10–12, *P. magellanicus* fresh tissue; lanes 13–18, *P. magellanicus* mixed storage.

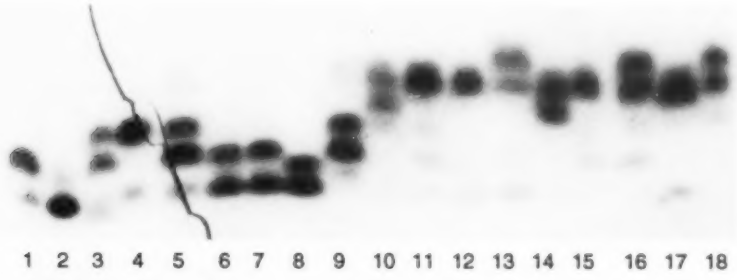

FIG. 3. Starch gel stained for phosphoglucomutase (PGM). Lanes 1–3, *C. islandica* fresh tissue; lanes 4–9; *C. islandica* mixed storage; lanes 10–12, *P. magellanicus* fresh tissue; lanes 13–18, *P. magellanicus* mixed storage.

because the *Chlamys* meats retain the band specific to themselves and this band is not found in the *Placopecten* tissue.

Glutamic oxaloacetate transaminase (GOT)

The allozyme bands are stronger staining in *Placopecten* (Fig. 2a). The band with a mobility of 61 is the most dominant and is characteristic of all *Placopecten* samples. The band with a mobility of 76 is the dominant band in *Chlamys*. These bands always occur in the species they characterize and never occur in the other species, i.e., they are diagnostic for the samples tested. Secondary bands were also observed in each species but they do not overlap with the diagnostic ones and they do not interfere with the diagnosis. The secondary band 100 occurs only in *Placopecten* after maintenance on ice or at −80°C. Mobilities 55 and 40 appear occasionally in *Chlamys*. There was no effect of pretreatment on the diagnostic power of bands 61 and 76. There is no transfer of GOT between meats of each species kept in contact with each other (Fig. 2b).

Phosphoglucomutase (PGM)

The diagnostic power of this enzyme is severely compromised by a high degree of intraspecific variation. In general the faster migrating bands belong to *Placopecten*, while the slower migrating bands belong to *Chlamys* (Fig. 3). The slower moving bands of *Placopecten* may coincide with the faster bands of *Chlamys*. In addition, there is leakage when the meats are held in close contact with each other. This enzyme cannot be used to provide a diagnostic assay.

DNA Sequencing and Restriction Digests

The 18S rRNA genes for *P. magellanicus* and *C. islandica* differ in 15 nucleotide positions over 1815 base pairs (Fig. 4). All differences were by substitutions as opposed to deletions or insertions. Nine of the substitutions were transitions (interchange of purines or pyrimidines) and six were transversions (change from purine to pyrimidine or vice versa). The differ-

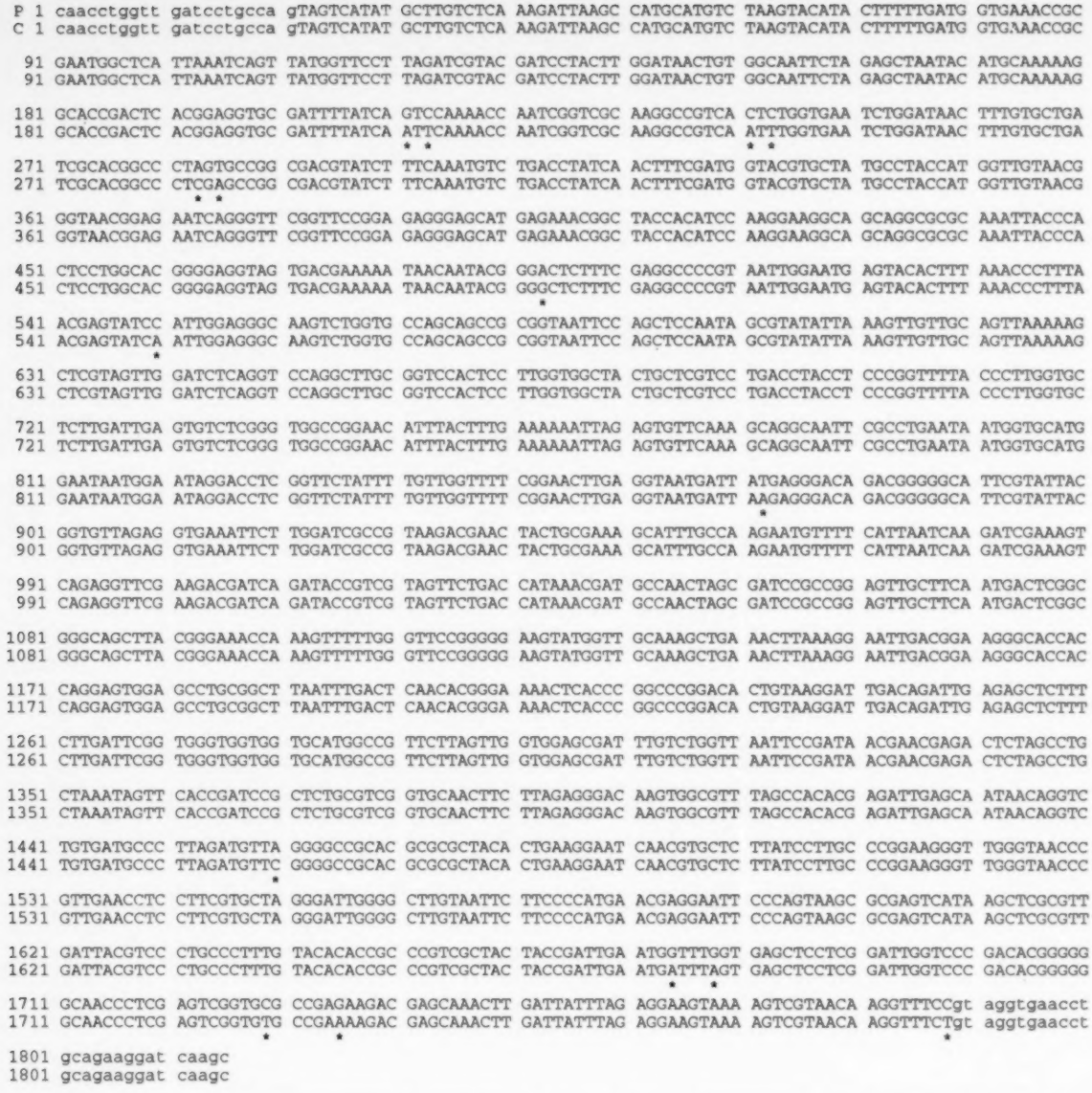

FIG. 4. Alignment matrix for 18S rDNA sequences of the sea scallop *P. magellanicus* (P) (upper sequence) and the Iceland scallop *C. islandica* (C) (lower sequence), constructed visually. Lowercase letters at the 5′ and 3′ ends of the gene designate nucleotide sequences of the amplification primers; asterisks indicate nucleotide differences between the sequences.

ences between the species can be detected by sequencing over the diagnostic regions alone.

Analysis of the sequences provided a number of diagnostic restriction enzymes that could be used to separate the species. Some of the restriction fragments are too small to record reliably on standard gels. One enzyme that digests the gene into diagnostic fragments is XHO I. In *Placopecten*, there is one internal site for this enzyme. Digestion releases two fragments (Fig. 5a), one of 1717 bases (1.7 kb) and one of 98 bases (0.98 kb). In *Chlamys*, there are two internal sites that this enzyme recognizes. Digestion releases fragments of 281, 1436, and 98 bases (Fig. 5a). The two larger species-specific fragments (1.7 and 1.4 kb) are readily separated by electrophoresis after only 2 h (Fig. 5b).

Discussion

In the present sample, allozyme electrophoresis yielded two enzymes, MPI and GOT, that were diagnostic under different pretreatment regimes and when meats of each species were held in contact for 12 d. However, after examining 1700 specimens of *C. islandica* from the northeastern Atlantic, Fevolden (1989) found that MPI could not be scored consistently. The reasons for this inconsistency are not clear; however, for enforcement

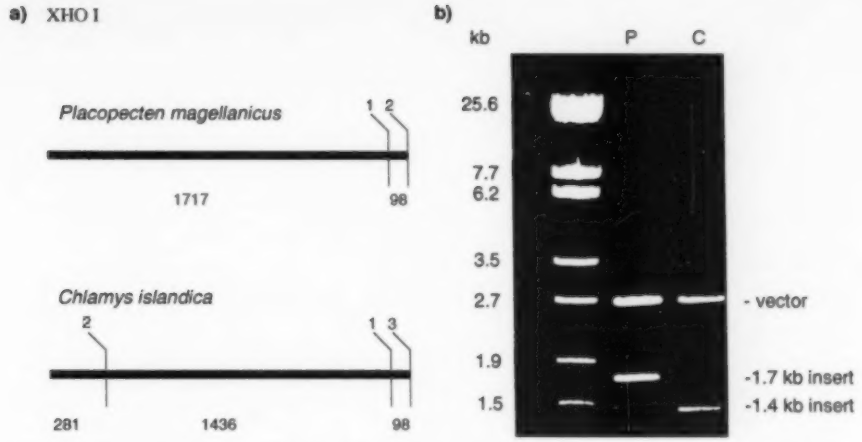

FIG. 5. (a) Restriction fragment lengths of 18S rDNA from *P. magellanicus* (upper) and *C. islandica* (lower) after digestion with the enzyme XHO I. Numbers indicate the number of base pairs in each fragment. (b) Corresponding agarose gel stained with ethidium bromide to identify DNA activity. The upper band is the plasmid vector; the lower band is the digested (XHO I) 18S rDNA gene insert for *Placopecten* (lane 2) and *Chlamys* (lane 3). The marker is lambda DNA digested by STY I (lane 1). Only the two larger fragments (1.7 and 1.4 kb, respectively) can be resolved on this gel.

purposes, samples often cannot be recollected, and if this enzyme system is used for species identification, it should be done so in combination with GOT. Fevolden (1989) also found PGM to be highly polymorphic and of low scoring value in *Chlamys*, as is reported here for both species. Foltz and Zouros (1984) scored GOT activity in juvenile *Placopecten* and found two bands appearing with high frequency (51 and 46%); in contrast, we observed only one common band in this species. This is probably the result of within-species geographic variation. Allozyme electrophoresis has potential in providing a diagnostic test for the identification of the two species. The MPI and GOT enzyme systems used in combination would appear to be the most useful, especially as an initial screening procedure. However, with the results of other workers in mind, it would not be prudent to declare these enzyme systems diagnostic without a further investigation of geographic differentiation, particularly of *C. islandica*. This study has established that the tissue does not have to be fresh or fresh-frozen in order to achieve good gel resolution, at least with these enzyme systems.

DNA analyses have provided two diagnostic approaches for separating the two species. The DNA can be sequenced using only one or two primers, or restriction enzyme digests can be used to discriminate between the species. A survey for geographic variation should also be completed for this approach to confirm the hypothesis of species-specific conservation in this gene for these species.

With the unequivocal diagnostic power of the DNA analyses established, further improvements could be made to the protocol to render it more amenable to routine large-scale surveys. Instantaneous freezing of the muscle is not necessary for successful extraction. DNA has been extracted from meats collected from a commercial sample of unknown age and stored on ice for a further 6 d prior to extraction (personal observation). Also, in this study, DNA was cloned into a plasmid vector prior to sequencing. This step is time-consuming and if only one or two sequencing reactions are required, the amplified DNA can be sequenced directly following the protocol of Bachman et al. (1990). We have done this routinely in the laboratory with very good results. Similarly, the amplified DNA can be digested directly with XHO I or one of the other diagnostic restriction enzymes and the fragments run out on a gel. This is the most cost- and time-effective way of establishing species identity using DNA analyses. Amplification thermocyclers can be built at low cost (Rollo et al. 1988) if commercial amplifiers are not available. Reactions can also be manually cycled through a series of heating blocks or water baths.

In conclusion, this study has shown that biochemical methods can be used to positively discriminate between the two species of scallops. The use of allozyme electrophoresis would require more study of intraspecific variation over the fishing areas before it could be said to be diagnostic in all cases. However, the use of DNA analysis appears to be diagnostic and can be applied by using either sequencing techniques or restriction enzyme digests. The time and costs involved are reasonable in comparison with the other costs involved in the successful prosecution of fishing regulation infractions. Modifications to the protocol and establishment of a dedicated facility for such testing could further improve costs and result in overall better management of the two scallop species in question.

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Using Hard-part Increment Data to Estimate Age and Environmental Effects

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Fish grow in response to the extrinsic effects of their biotic and abiotic environment and in accordance with the intrinsic effects of size or age already attained. In this paper, a family of linear models are proposed that assume that growth, as reflected in increments in scales or other bony structures, can be divided into intrinsic age or size effects and external environmental effects. The models presented are of varying complexity. Some of the models use data from annular rings on bony parts, some use length or size attained, and some use both. Generalization to nonlinear or generalized linear models is indicated. Methodology for comparing subsamples of fish, based on sex, condition, trapping method, or any other identifiable characteristic, is discussed. The models are applied to several samples of smallmouth bass (*Micropterus dolomieu*) collected during 1988–90.

Les poissons se développent en réaction aux effets extrinsèques de leur environnement biotique et abiotique et selon les effets intrinsèques de la taille ou de l'âge qu'ils ont déjà atteints. Dans cette étude, on propose une famille de modèles linéaires fondés sur l'hypothèse selon laquelle la croissance, observée dans l'accroissement des écailles ou d'autres structures osseuses, peut se séparer en effets intrinsèques de l'âge ou du sexe et en effets externes de l'environnement. Les modèles présentés sont de complexité variable. Certains utilisent les données sur les anneaux de croissance des structures osseuses, d'autres font appel à la longueur ou à la taille atteinte et certains utilisent les deux. On indique s'il y a généralisation à des modèles non linéaires ou à des modèles linéaires généralisés. La méthodologie de comparaison des sous-échantillons de poissons, à partir du sexe, de l'état biologique, de la méthode de capture ou de toute autre caractéristique identifiable, est analysée. Les modèles sont appliqués à plusieurs échantillons d'achigan à petite bouche (*Micropterus dolomieu*) recueillis en 1988–1990.

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The purpose of this paper is to provide a family of models to model fish growth using a few easily interpretable parameters and to allow for simple and straightforward comparison of fish stocks, gear types, sex effects, management strategies, and so on. Linear model theory is used in most of the development, so standard analysis of variance tables and tests are available. For the most important models, standard linear model regression programs can be used for the computations; for the more complex models, special-purpose code may be required.

Fish grow in response to their biotic and abiotic environment and in accordance with some function of size or age already attained. A record of the growth history of an individual fish is contained in the increments between annuli on scales and other bony structures. The traditional method of extracting this information is through backcalculation (Lee 1920; Hile 1943; Whitney and Carlander 1956; Southward 1962; Ricker 1973, 1975) in which the information in the bony part is used to estimate length in previous years (see also Smith 1987). Noakes and Campbell (1992) used annular increments on geoduck clam shells to model environmental factors; their methodology is different from that suggested here.

The models discussed in this paper generalize the methodology given by Weisberg (1986) and Weisberg and Frie (1987) removing the limitation in those papers to a single sample from a population. First, the most basic model, using only scale increments, is described and illustrated. Some of the possible elaborations of the basic model are then given. This is followed by a largely pedagogical discussion of making comparisons between samples, years, and so on. The next section presents another variation of the model in which only length given age is used in the estimation; scale increments are not required. This method requires several years of data and is likely to produce estimates that are more highly variable than scale models, because of collinearity problems. Finally, an analysis based on a combination of scale and length data, as is done in backcalculation, is presented. Conclusions and further comments on the methodology proposed complete the paper.

All examples will refer to data collected as part of two Minnesota Department of Natural Resources fisheries research projects on smallmouth bass (*Micropterus dolomieu*) in West Bearskin Lake, Minnesota, by G. Siesennop, J. Hirsch, and T. Sak. The data consist of five samples collected over three years using two different methods of capture, as described in Tables 1 and 2. The models divide growth into two components, one due to age effects and one due to year or environmental effects. In addition, we may wish to see if measured growth varies by the collection method, trapnetting versus electrofishing, and if measured growth varies from sample to sample. To simplify the presentation in this paper, the fish from

TABLE 1. Sample sizes for the Beakskin Lake data.

Year and method	Sample size
1988 spring electrofishing	30
1989 spring electrofishing	65
1990 spring electrofishing	181
1988 fall trapnetting	67
1989 fall trapnetting	102
Total	441

TABLE 2. Distribution of fish by year-class.

Year-class	Number of fish
1989	3
1988	39
1987	150
1986	18
1985	1
1984	32
1983	177
1982	9
1981	11
1980	3
1978	1
1977	1

the 1977–82 age-classes for which little data are available will be excluded from all analyses, although this exclusion is not required for fitting the models.

Methods

Scale Increment Models

We assume that growth in a given year can be divided into two major components: a component due to the age or the size of the fish and a component due to environmental factors. As most fish grow, they lay down annular growth rings on their scales. A key scale from each of a sample of fish can then be removed, and the distances between the annular rings can then be measured (e.g., see Frie 1982). We assume that the distance between annuli is proportional to growth; if this distance is large, growth is large; if it is small, growth is less. Each fish contains a record of its own growth history in the interannuli distances. In addition, we assume that the age of the fish is known accurately by counting annuli and do not here consider the effects of incorrect aging.

The purpose of growth modelling is to combine the growth history contained in the scales of many fish into a few descriptive parameters that capture the important features of growth of a "typical" fish in a given year from a given year-class. Simple models are preferred as long as the resulting fitted model can reproduce the data with reasonable accuracy. A useful class of models assume that growth depends on an intrinsic factor, such as age or size of a fish, plus an external factor that we will call the "year" effect. Lumped into the year effect are all manner of environmental, management, and data collection practices that apply to all fish in the population in a given year. The age effects characterize growth if the external effects are held fixed. In most fish stocks, the age effect will decrease with age, as younger fish generally grow faster than do older fish; this can be modelled by fitting appropriate curves to the estimated age effects. The year effects characterize year to year variation and may help understand the effects on a fish stock of changes in the environment or in managemen practices.

The most elementary model assumes only additive age and year effects, given by the equation

$$\text{(1)}\quad \text{Expected growth increment} = \text{Age effect} + \text{Year effect}$$

where for now a growth increment is the radial scale increment for the given growth year. When appropriate, this model is highly desirable because the effects of each age and of each year are summarized by a single number. Unfortunately, one cannot expect this model to be adequate in all situations. Implicit in (1) is the assumption that the effect of the environment is the same for all age-classes; the possibility that, for example, habitat in a given year may be better for young fish than for older fish is excluded by (1). When it is necessary to include this possibility, model (1) can be expanded by including an interaction:

$$\text{(2)}\quad \text{Expected growth increment} = \text{Age effect} + \text{Year effect} + \text{Age} \times \text{Year interaction.}$$

The fit of models (2) and (1) can be compared via a hypothesis test. Should model (1) prove untenable for all the data, it may be adequate for a subset of the data. For example, if samples are taken with size-selective gear that rarely capture longer, and hence older, fish, then fitting model (1) to all the data may be inadequate because the rare older fish in the sample will typically be slower growing than average for their age-class, and this will be reflected in the presence of age by year interactions. Similarly, size-selective mortality can be reflected in significant interactions. However, model (1) may be appropriate if the older age-classes are excluded from the analysis.

Both models (1) and (2) represent standard linear models for expected growth, so one might hope that standard software could be used to fit and compare these models and to summarize results. Whether or not this is possible depends on assumptions concerning the statistical errors. We assume that fish in the samples are all statistically independent, which will generally hold at least approximately as long as the number of sampled fish is a small fraction of the entire fish stock. Independence in this setting roughly means that the prediction of the deviation from average growth of any specific fish in the sample is not improved by knowing the actual growth of the other fish in the sample.

We must then make assumptions about correlations between growth increments for the same fish. The assumption of *general correlation structure* allows arbitrary correlations, while *identity correlation structure* assumes that the increments within a fish are uncorrelated with constant variance. For the general correlation, nonstandard computational methods can be derived using the results on unbalanced growth curves in Jennrich and Schluchter (1986) as summarized in the Appendix. For identity correlation, the computations are greatly simplified and can be done using standard statistical software.

Finally, we assume that the errors are normally distributed, and hence use standard linear model theory. For the identity correlation case, normality is needed only to get distributions for test statistics; in the general correlation case the normality assumption is more critical. See Smith (1987) for a discussion of the assessment of normality.

In the identity correlation case, the computations can be done as if the basic unit of analysis were a single scale increment.

TABLE 3. Average scale increments.

Year-class	Growth year	Age	Number of fish	Scale increments	
				Average	SD
1983	1983	1	177	1.504	0.347
1984	1984	1	32	1.483	0.336
1985	1985	1	1	2.113	0
1986	1986	1	18	1.136	0.217
1987	1987	1	150	1.316	0.281
1988	1988	1	39	1.463	0.200
1989	1989	1	3	1.526	0.048
1983	1984	2	177	1.474	0.398
1984	1985	2	32	1.163	0.380
1985	1986	2	1	0.599	0
1986	1987	2	18	1.798	0.341
1987	1988	2	131	1.348	0.285
1988	1989	2	23	1.287	0.202
1983	1985	3	177	1.034	0.310
1984	1986	3	32	1.093	0.242
1985	1987	3	1	0.652	0
1986	1988	3	16	1.604	0.690
1987	1989	3	84	1.324	0.257
1983	1986	4	177	1.095	0.362
1984	1987	4	32	1.265	0.391
1986	1989	4	7	1.191	0.331
1983	1987	5	177	1.264	0.534
1984	1988	5	24	1.127	0.295
1983	1988	6	119	1.261	0.396
1984	1989	6	14	1.122	0.306
1983	1989	7	48	1.159	0.389

Thus for example, a 10-yr-old fish in a sample contributes 10 separate increments to the analysis, while a 3-yr-old fish contributes 3 units; current season's growth, the distance from the last annular increment to the edge of the scale, is ignored for modelling using only scales. To make use of all complete data, fish captured at the end of a current growing season should be classified as being caught at the beginning of the following growing season. To each scale increment, we can assign the age of the fish and the year in which the growth took place; these are the levels of the factors "age" and "year", respectively. These data can then be input to a standard regression or analysis of variance program, such as SAS-GLM. In model (1), we have purposely excluded a term for the overall mean. If age effects are fit before year effects, this forces the program to estimate a coefficient and standard error for each age and makes the parameters easier to interpret. A special-purpose program for this analysis is available, as described in the Discussion section.

In the identity correlation case, the F-test for interaction in the fit of (2) provides the basic diagnostic for comparing model (1) with model (2) (Weisberg 1985, section 4.3). In the general correlation case, several options are available for this comparison. Since little is known about the distribution of the resulting test statistics, use of the test for the identity correlation case is recommended even for the general correlation case until further research suggests a better procedure.

The above models can be illustrated on the West Bearskin Lake data, which is summarized in Table 3. Using the identity correlation model, one can fit using average scale increments for each year-class/age group as the response and the number of observations per mean as weights. When model (1) is fit in to the averages in Table 3, the "residual" computed by the program will be the "interaction" shown in the Table 4. The correct residual, as shown in Table 4, is given by

$$\text{Residual sum of squares} = \Sigma(n - 1)\text{SD}^2$$

$$\text{Residual df} = \Sigma(n - 1)$$

where the SD are the standard deviations and the n are the number of fish shown in Table 3. The estimate of error variance is about 0.13, and the F-test for interaction is $F = 4.63$ with (13, 1684) df, so the p-value is ≤ 0.001, suggesting that model (1) is not completely adequate.

TABLE 4. Analysis of variance for models (1) and (2), West Bearskin data, 1983–89 age-classes.

Source	df	SS	MS	F	p-value
Age	6	25.71	4.29	32.31	0
Year	6	13.93	2.32	17.50	0
Interaction	13	7.98	0.61	4.63	0.000
Residual	1684	223.38	0.13		

TABLE 5. Coefficient estimates for the example fitting model (1) with both uncorrelated and correlated errors.

Coefficient	Increments uncorrelated		Increments correlated	
	Estimate	SE	Estimate	SE
Age 1	1.30	0.05	1.26	0.04
Age 2	1.34	0.04	1.31	0.04
Age 3	1.30	0.04	1.30	0.03
Age 4	1.25	0.05	1.21	0.04
Age 5	1.18	0.05	1.15	0.04
Age 6	1.19	0.05	1.18	0.04
Age 7	1.16	0.05	1.13	0.05
Growth year 1983	0.21	0.06	0.25	0.05
Growth year 1984	0.14	0.05	0.16	0.05
Growth year 1985	−0.25	0.04	−0.25	0.04
Growth year 1986	−0.16	0.05	−0.14	0.04
Growth year 1987	0.07	0.05	0.11	0.03
Growth year 1988	0.06	0.05	0.09	0.03
Growth year 1989	Not estimable		Not estimable	

Before examining the interactions, we examine the fitted parameters for model (1) as if the interactions were unimportant. These are listed in Table 5 and plotted in Fig. 1 and 2. The figures give the coefficient estimates, joined by a solid line, and two hatched lines drawn at 2 standard errors to give a visual assessment of precision. We see that growth is generally decreasing by about 0.1 unit (millimetres of scale width) per year of age. The year effects are interpreted a bit differently, since a coefficient for the most recent year in the sample, 1990 in these data, is not estimated but is shown as "not estimable" in the table. All the year effects must be interpreted relative to the year 1990; think of the most recent year as having effect equal to 0. Some computer programs may resolve the overparameterization of the model in other ways. We get the interesting result that the early years, 1983 and 1984, were better than 1990, the next two years, 1985 and 1986, were worse years, and the remaining years were about the same as 1990. The coefficient for the worst year, 1985, is about equal to twice the size of an average growth increment for any age: 1985 seems to have been a bad year indeed. From Table 1 we also see that only one fish from the 1985 age-class has been

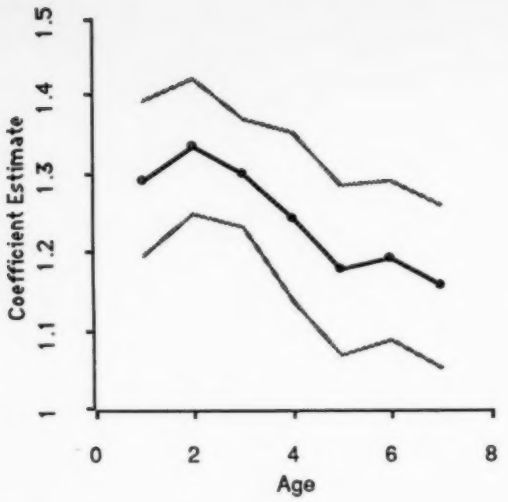

FIG. 1. Age effects for the West Bearskin Lake smallmouth bass data, all samples, 1983–89 year classes.

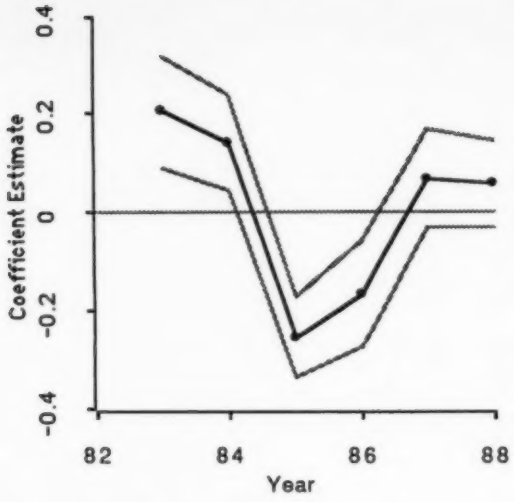

FIG. 2. Year effects for the West Bearskin Lake smallmouth bass data, all samples, 1983–89 year-classes.

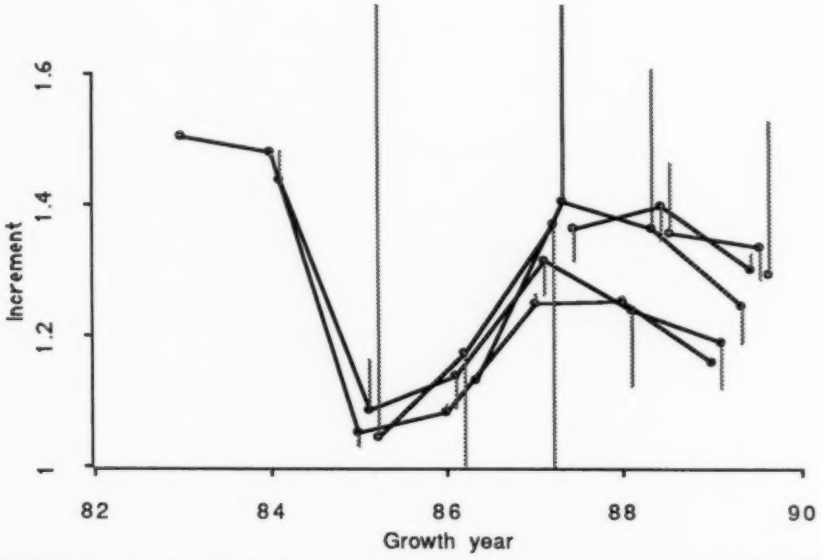

FIG. 3. Growth plot by year-class. For each year-class, the estimated growth increments from the fit of (1) are joined by a solid line. The vertical hatched lines join the observed growth increments to the fitted increments. Long lines correspond to poor fit by the model.

captured in the five samples to date, giving an additional suggestion of environmental problems in 1985.

We now turn to the interactions. Numerical values for the interaction parameters are of little use because the estimable parameters depend on the combination of year-classes and ages at capture in the data and on the computer program. We propose a graphical examination, as shown in Fig. 3. In this figure, the fitted scale increments (age effect + year effect) are joined by a solid line for each year-class. The vertical hatched lines join the observed growth increments, that is, the observed averages, to the fitted values. Long lines correspond to averages that are poorly fit by the model and are indicative of interactions. The longest lines are for the 1985 and 1986 year-classes, suggesting that not only were these years bad generally for growth, but the fish born in these years do not have growth histories that are comparable to with those of other age-classes. One option at this point is to refit, excluding the 1985 and 1986 age-classes. The goal of the refitting is not to exclude these year-classes, but to understand the interactions. Without these two years, the p-value for interaction becomes 0.054, just marginally significant. The interaction plot is shown in Fig. 4. The fit of the simple model (1) to the reduced data now seems to be quite

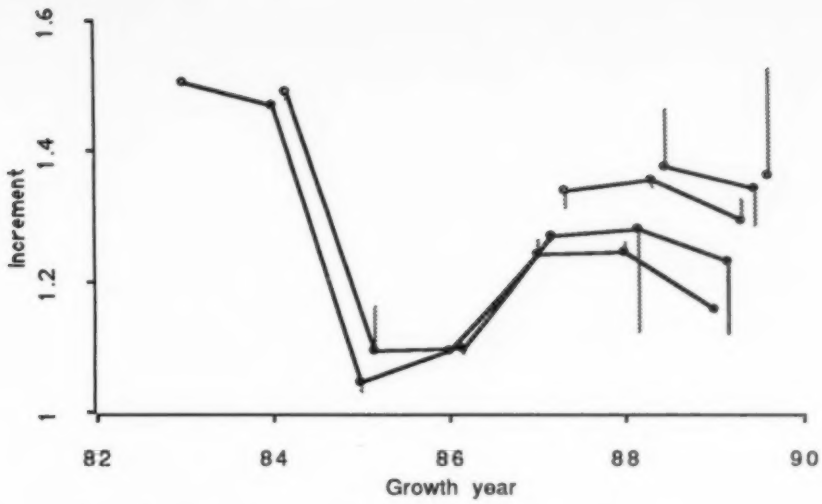

FIG. 4. Growth plot by year-class, with the 1984 and 1985 year-classes removed.

TABLE 6. Estimated covariance matrix between growth increments, West Bearskin Lake data.

	Increment						
Increment	1	2	3	4	5	6	7
1	1.00	0.36	0.25	0.18	0.12	−0.08	−0.09
2	0.36	1.00	0.44	0.55	0.30	−0.05	0.03
3	0.25	0.44	1.00	0.50	0.50	0.11	−0.10
4	0.18	0.55	0.50	1.00	0.61	0.08	0.03
5	0.12	0.30	0.50	0.61	1.00	0.10	−0.04
6	−0.08	−0.05	0.11	0.08	0.10	1.00	−0.01
7	−0.09	0.03	−0.10	0.03	−0.04	−0.01	1.00

adequate. In comparing Fig. 3 and 4, we see that the overall trends are the same, with 1985 and 1986 identified as poor years for all age-classes, and that fish from the 1985 and 1986 year-classes are different from fish in other year-classes. We attribute the interaction to the difference between 1985 and 1986 year-classes and the other year-classes.

For comparison, we have fit model (1) using an arbitrary correlation structure. The estimated correlation matrix between the scale increments is given in Table 6, and the standard deviations of the increments are estimated to be 0.30, 0.34, 0.32, 0.35, 0.50, 0.39, and 0.39, respectively, for increments at age 1, 2, . . ., 7, suggesting that very little is lost by the assumption of common standard deviation which was previously estimated to be $0.131^{1/2} = 0.36$. The fitted parameter estimates are given in Table 5; clearly there is little to differentiate the fit with the arbitrary correlation matrix from the fit with the identity covariance matrix. We expect this to hold true in most cases because this model is based on increments, not on attained size, and increments tend to be much less correlated (Box 1950).

Subsetting

As with any linear model, hypotheses can be explored by adding parameters to a model and performing relevant F-tests. This procedure may not be practically feasible, particularly in larger problems. An alternative that produces numerically and theoretically the same answers is called *subsetting*. The idea is to fit the same model to subsets of the data and then combine answers to get tests. The details are laid out in Weisberg (1985, section 7.4). Since the basic idea may not be familiar to many readers, we demonstrate the method by example.

TABLE 7. West Bearskin Lake smallmouth bass, 1983–89 age-classes.

	Combined		Electrofishing		Trapnetting	
Source	df	SS	df	SS	df	SS
Age	6	25.71	6	13.78	5	14.15
Year	6	13.93	6	10.75	5	3.51
Interaction	13	7.98	10	3.59	9	4.98
Residual	1684	223.38	1065	107.10	602	109.76

Consider testing the hypothesis that the electrofishing samples are different from the trapnetting samples. To perform the test, fit the model to the electrofishing data alone, to the trapnetting data alone, and to all data combined. This is summarized in Table 7, whch gives the sums of squares and degrees of freedom for the three fits. Add together the sum of squares for Age and for Year for electrofishing and trapnetting, as on line 3 of Table 8; this is the sum of squares due to fitting separate models for electrofishing and trapnetting. Line 4 of Table 8 is the sum of Age and Year sum of squares for fitting the combined data. The difference between lines 3 and 4 is the additional sum of squares explained by fitting separate models. Similarly, the additional sum of squares for interaction for fitting separate models is the difference between the interactions for the separate model and the interaction for the combined model. This mean square is used as the denominator for the F-test for comparing the combined with the separate model, $F = 0.25/0.10 = 2.50$, with (10, 6) df, p-value = 0.13, and there is no evidence of the need for separate models for electrofishing samples and trapnetting samples. This general method can be used for any comparison due to gear type, samples, sex, state of maturity, and so on.

When fitting with a general correlation matrix, tests must be based on sum of squares and cross-product matrices, the mul-

TABLE 8. Comparing electrofishing and trapnetting, 1983–89 age-classes.

	Model	df	SS	MS
(1)	Electrofishing Age + Year	12	24.53	
(2)	Trapnetting Age + Year	10	17.65	
(3)	(1) + (2)	22	42.18	
(4)	Age + Year, combined	12	39.64	
(5)	Due to separate models, (3) − (4)	10	2.54	0.25
(6)	Electrofishing + trapnetting interaction	19	8.57	
(7)	Interaction, combined	13	7.98	
(8)	Remaining interaction, (7) − (6)	6	0.59	0.10
(9)	Residual	1667	216.86	0.13

tivariate analogs of the test described above. However, nothing is known about the statistical properties of such tests for data like those available for scales analysis.

Modelling Growth from Lengths

Each year, a fish increases in length. Its length at age 5, say, is the sum of its length increases during the first 5 yr of life. Lengths alone are not usually thought of as adequate for modelling growth because we cannot disentangle length increments using a single fish. However, if fish from the same age-class are collected in a number of years, and several age-classes are available, it may be possible to estimate age and year coefficients from lengths alone.

As with scales, we assume that length increments can be modelled via equation (1), now interpreting a growth increment to be an increase in length. Since an observed length is just equal to the sum of the length increments in the previous years, we have the model

$$\text{(3)}\quad \text{Expected observed length} = \Sigma\ \text{Age effects} + \Sigma\ \text{Environmental effects}$$

and this model can be fit via linear regression models. This leads to modelling that is identical in interpretation to that based on scales. However, the model (3) will generally lead to collinearity problems, resulting in estimates that are highly variable.

If the fish were not captured at the very beginning or the very end of the growing season, a correction for lengths is required to account for current season's growth before (3) can be fit. A simple corrected length is given for each fish by

$$\text{(4)}\quad \text{Observed length} \times 1 - \left(\frac{\text{Scale increment from last annulus to edge}}{\text{Total scale radius}}\right)$$

and (4) is used in the following example.

For the West Bearskin data, enough year-class/capture-year combinations are observed to estimate six age effects, six year effects, and three age by year interactions. For these data, the F-test for interaction is $F = 1.23$, with 4, 404 df, giving a p-value of about 0.3, suggesting no evidence against an additive model. Coefficient estimates for the additive model are given in Table 9. The estimated growth for age 1 is 71 mm with a standard error of 4 mm; for the other years of age the estimated growth is close to 40 mm per year. The environmental effects are relatively small, on the order of only a few millimetres per year, except for 1984 which had a negative effect, relative to the standard year of 1989 of about −34 mm, roughly comparable to a year's growth. This analysis does not completely agree with the analysis based on scales and reported in Table 5, particularly with regard to the environmental components.

TABLE 9. Growth model based on lengths and lengths and scales, 1983–89 age-classes, West Bearskin Lake.

	Length model		Length/scale model	
Coefficient	Estimate	SE	Estimate	SE
Age 1	71.0	4.0	63.9	9.2
Age 2	49.1	4.2	44.5	6.0
Age 3	43.7	2.3	45.9	6.8
Age 4	39.3	5.2	44.0	6.7
Age 5	42.9	4.3	45.0	7.7
Age 6	27.8	3.1	45.4	4.9
Age 7	42.8	1.4	44.4	2.8
Year 1983	−2.9	2.2	−9.9	4.7
Year 1984	−34.1	13.1	−57.4	16.3
Year 1985	−1.3	12.4	24.6	16.6
Year 1986	5.6	3.8	−10.1	6.8
Year 1987	−7.2	3.2	−4.0	7.3
Year 1988	−7.4	3.3	2.6	8.0
Year 1989	Not estimable		Not estimable	

In fitting (3) to length data, the same technical assumptions that were made for the scale data are used. In particular, we assume that yearly increments in length are independent between fish, that successive increments in a single fish are independent, and that variability in increments is constant over years. Since no individual fish is observed more than once, a general covariance matrix of length increments within a fish cannot be estimated, although one could modify the method described in the Appendix with the covariance matrix diagonal, but not necessarily a multiple of the identity. For making inference statements, we further assume length increments to be normally distributed.

Combining Scale and Length Analyses

A classical approach to modelling fish growth is through the use of Fraser-Lee "backcalculation" to use scales to apportion length to previous years (e.g. see Bagenal and Tesch 1978). In Weisberg (1986, section 3), an alternative to backcalculation that combines both scale and length data was proposed for a single sample of fish; that methodology can be extended to multiple samples. For simplicity, we again assume the identity correlation case and ignore any current season growth, assuming that lengths have been adjusted.

Consider a specific growth year for a specific fish, and let the pair (x, y) represent the pair (scale increment, length increment) for that fish in that year. Although y is not observable, we can still make assumptions concerning (x, y), given symbolically by

$$\text{(5)}\quad \begin{pmatrix} x \\ y \end{pmatrix} \sim \mathrm{N}\left(\begin{pmatrix} \mu \\ \eta \end{pmatrix}, \begin{pmatrix} \sigma_x^2 & \rho\sigma_x\sigma_y \\ \rho\sigma_x\sigma_y & \sigma_y^2 \end{pmatrix}\right)$$

where the means μ and η are specified by equation (1) to be sums of age and year effects, the variances of x and y are assumed constant for each fish and each growth year, and the

correlation ρ between x and y is also assumed constant. Each pair (x,y) is independent of all other pairs.

Equation (5), with the independence assumptions following it, is the basis for the combined analysis; the normality makes the computations straightforward. Let $L = \Sigma y$ be the observed length of a given fish, and let S be the observed vector of all the scale increments for a given fish; the length of S is equal to the age a of the fish at capture. From (5), it is easy to derive the joint distribution of S and L:

$$(6)\quad \begin{pmatrix} S \\ L \end{pmatrix} \sim N\left(\begin{pmatrix} \mu \\ \Sigma\eta \end{pmatrix}, \begin{pmatrix} \sigma_x^2 I & \rho\sigma_x\sigma_y 1 \\ \rho\sigma_x\sigma_y 1 & a\sigma_y^2 \end{pmatrix}\right)$$

where μ is the now a vector whose elements are the means for each growth increment, I is the $a \times a$ identity matrix, and 1 is the a-vector of ones. The conditional distribution of L given S is

$$(7)\quad L|S \sim N(\beta_0 + \beta(\Sigma S), a\sigma^2_{y|x})$$

where in (7), a number of new parameters have been defined in terms of the parameters of (6): $\beta = \rho\sigma_y/\sigma_x$ is a slope parameter that is the *same* for every age-class, which can usefully be thought of as a scale factor for converting scale radii to lengths, $\beta_0 = \Sigma\eta - \beta\Sigma\mu$ is an intercept that will be *different* for each year-class and age at capture, and $\sigma^2_{y|x} = \sigma_y^2(1-\rho^2)$. Thus, in the regression of L on S, model (7) specifies that each group of fish of the same year-class and year of capture should have a linear regression of length on total scale radius excluding current season's growth. The regressions should all be parallel, but have different intercepts; weighted least squares with weights equal to a, the age at capture, is required. A diagnostic test of model (3) is a test to see if the within-group regressions are parallel; the F-test for this is in Weisberg (1985, section 7.4).

Maximum likelihood estimates of the original parameters, age and year effects for both scales and for lengths, can then be computed using the following algorithm. First, fit the model (1) to the scale data only. These estimates can be used to get the estimates of the μ parameters. Next, use the length data only to fit the linear regression of L on S, as specified by model (7). This will give estimates of the β parameters. Finally, invert the equations following equation (7) that relate the age and year parameters for lengths to the βs to get the maximum likelihood estimates of the age and year parameters for lengths. Typically, at least 3 yr worth of data is required for these equations to be invertible. If all the parameters of the marginal length analysis described previously, except for the current season year effect, are estimable, then the equations are invertible. If they are not invertible, Weisberg (1986, section 3.2) suggested a method for getting unique estimates; however, the reliability of this method in general is not known.

For the West Bearskin data, the test for equal slopes is $F = 4.15$, df = 14,389, with a very small p-value. For these data, the model (7) is not appropriate, so the lengths and scales cannot be simply combined. For completeness, however, we give the coefficient estimates when these are computed in the last columns of Table 9. These estimates are similar to those obtained from the length only analysis.

Discussion

The main point of this paper is to demonstrate that it is possible to separate the effects of an intrinsic factor like size or age and external factors like environment and management strategies using fairly standard linear models. Qualitatively similar models can be fit to annular increments alone, to lengths alone, or to both annular increments and lengths combined. The models for annular increments are the simplest and are likely to be the most useful in practice. Data from lengths will in most instances lead to highly collinear models and consequently very poor estimates of parameters. The combined analysis is likely to have precision between the scale-only analysis and the length-only analysis.

All the models used assume that scale increments or increments apparent on other bones should indeed reflect growth. Of course, a fish has many scales, and the growth history recorded on one scale will be different from that recorded on another scale; indeed, since scales are rarely round, different measurements on the same scale will give a different growth history. One way to reduce this variability is to measure more than one scale per fish, or take more than one measurement per scale. The results contained in Newman and Weisberg (1987) for brown trout (*Salmo trutta*) suggest that two scales per fish might be highly desirable. These values can then be averaged for each fish before the model is fit.

Two approaches to treating growth within a fish are presented: the identity correlation case, in which growth increments within a fish are modelled to be uncorrelated and equally variable, and a general correlation case. The former has the advantage of easy computations and allows for the use of standard computer programs. The latter method allows for estimation of parameters, but other issues like comparison of subsamples and testing for lack of fit cannot be easily addressed with the current state of knowledge. We have shown by example that the two sets of assumptions lead to the same qualitative conclusions; we expect this to be generally true, and thus we have faith in the analysis produced by the identity correlation case, even though the assumptions are incorrect.

One of the troubling features of the no-interaction models is that they will rarely fit: given generally very large samples, interactions will almost certainly be found to be "significant" even though the interactions are small relative to the main effects. In this case, the main effects will capture most of the information. The description will not be perfect, but it will be useful nonetheless. Graphical examination of the interactions can give fine details about specific years or age-classes and can often lead to finding subsets of the data where the model fits more successfully.

Many other models retain the division of growth into intrinsic and external components, but combine these quantities in a different way. For example, a multiplicative model is given by

$$\text{Expected growth increment} = \text{Age effect} \times \text{Year effect}$$

and total growth is then the product of the individual growth increments, rather than the sum. This model assumes that percentage changes are constant rather than absolute changes, allowing for systematic differences in age and particularly year effects for different sizes of fish. Taking logarithms:

$$\log(\text{Expected growth increment}) = \log(\text{Age effect}) + \log(\text{Year effect})$$

showing that the multiplicative model is equivalent to model (1) except using log(growth) as the response. Besides allowing for certain types of interaction, this model also allows for non-constant variance, as it requires that the variability in growth be proportional to expected growth.

Another alternative is to replace the intrinsic age effects by size effects and fit a model of the form

$$\text{Expected growth increment} = f(\text{Size}) + \text{Year effect}$$

where size refers to some measure of size and the growth function $f(\cdot)$ is an unspecified growth function, for example the (derivative of the) von Bertalanffy (1957) or some other growth function. This model is nonlinear if $f(\cdot)$ is nonlinear. To retain linearity, one could discretize "size" into a few nonoverlapping size-classes and then fit the model

$$\text{Expected growth increment} = \text{Size-class effect} + \text{Year effect}$$

which can now be fit as was done for model (1). This will permit estimating a coefficient for each size-class, eliminating the need to estimate a growth function, and allowing the fit of a linear model in place of a nonlinear model.

Computer Program

A special-purpose program for Macintosh, PC, and Unix workstations has been prepared that can fit all the models discussed in this paper and is available for a nominal fee from the Minnesota Sea Grant Institute, University of Minnesota, St. Paul, MN 55108.

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Appendix

Model (1) or (2) can be fit to scale data with an arbitrary covariance matrix using a general approach due to Jennrich and Schluchter (1986). Consider fish of year-class c. Fish from this year-class could have been captured in several different years. Suppose the ages at capture are given by a set A_c. Let x_{caj} be the $a \times 1$ vector of scale increments for the jth fish of year-class c captured at age a. Suppose there are n_{ca} such fish. Assuming that the increments are normally distributed, we have

$$x_{caj} \sim N(D_{ca}\theta, \Omega_a)$$

where Ω_a is the leading $a \times a$ submatrix of the covariance matrix between yearly growth increments Ω that is common to all year-classes, θ is a vector of age effects, year effects, and possibly interactions of length, say p, and D_{ca} is an $a \times p$ known-design matrix of ones and zeroes, such that D_{c1} is the first row of D_{c2}, D_{c2} is the first two rows of D_{c3}, and so on. For example, for the additive model with age and year effects, suppose that the ages in the sample are 1 to a maximum of $m = 7$, and the years in the sample are 1983–89. Then the number of parameters is $p = 13$, with the first seven components of θ giving the age effects and the last six the year effects (the year effect for the last year is not estimable, so we do not include it in the D-matrices). The matrix $D_{1986,4}$, for the 1986 year-class, is a 4×14 matrix:

$$D_{1986,4} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

and, for example, $D_{1986,2}$ is the first two rows of this matrix.

Given normality, the log-likelihood for Ω and θ can be written as

$$\text{(A.1)}\quad L(\theta,\Omega) \propto -\frac{1}{2}\sum_c \sum_{a\in A_c} n_{ca} \log|\Omega_a| - \frac{1}{2}\sum_c \sum_{a\in A_c} \sum_j (x_{caj} - D_{ca}\theta)^T \Omega_a^{-1} (x_{caj} - D_{ca}\theta).$$

Jennrich and Schluchter (1986) provided an algorithm for maximizing (A.1), which consists of first estimating θ given the current estimate of Ω, then estimating Ω given the current estimate of θ, and repeating until convergence. For the problem at hand, there is considerable simplification when we assume that each scale has measurements for all ages 1 up to the age at which the fish was captured. This excludes, for example, samples of wild-born and hatchery-born fish in which the first few years of growth for the hatchery-born fish are not of interest.

This case can be handled using the methods in Jennrich and Schluchter (1986) but not using the following simplifications.

Let $\bar{x}_{ca} = \Sigma_j x_{caj}/n_{ca}$ be the mean increments for all year-class c fish captured at age a. Also, define $S_{ca} = \Sigma(x_{caj} - \bar{x}_{ca})(x_{caj} - \bar{x}_{ca})^T/n_{ca}$ to be the matrix of corrected sums of squares and cross products; this matrix is free of all parameters. Then the log-likelihood can be rewritten as

$$\text{(A.2)}\quad L(\theta,\Omega) \propto -\frac{1}{2}\sum_c \sum_{a\in A_c} n_{ca} \log|\Omega_a| - \frac{1}{2}\sum_c \sum_{a\in A_c} n_{ca}\,\text{trace}(\Omega_a^{-1} S_{ca}) - \frac{1}{2}\sum_c \sum_{a\in A_c} n_{ca}\,(\bar{x}_{ca} - D_{ca}\theta)^T \Omega_a^{-1} (\bar{x}_{ca} - D_{caj}\theta).$$

Given an estimate $\tilde{\Omega}$ of Ω, the estimate of $\tilde{\theta}$ of θ is computed by generalized least squares from

$$\text{(A.3)}\quad \tilde{\theta} = \left(\sum_c \sum_{a\in A_c} n_{ca} D_{ca}^T \tilde{\Omega}_a^{-1} D_{ca}\right)^{-1} \left(\sum_c \sum_{a\in A_c} n_{ca} D_{ca}^T \tilde{\Omega}_a^{-1} \bar{x}_{ca}\right).$$

Given $\tilde{\theta}$, we can reestimate Ω, using the residuals. Let $r_{caj} = x_{caj} - D_{ca}\tilde{\theta}$. We need to "fill in" r_{caj} to have m elements, where m is the maximum age of all fish in the data. This is done by computing

$$r^*_{caj} = \begin{pmatrix} I \\ \tilde{\Omega}_{21}\tilde{\Omega}_a^{-1} \end{pmatrix} r_{caj}$$

where $\tilde{\Omega}_{21}$ is the $(m - a) \times a$ submatrix of $\tilde{\Omega}$ in the last $m - a$ rows and first a columns of $\tilde{\Omega}$. Next, if $\tilde{\Omega}_{22}$ is the last $(m - a) \times (m - a)$ submatrix of $\tilde{\Omega}$, compute a covariance correction matrix, R_a, given by

$$R_a = \begin{pmatrix} 0 & 0 \\ 0 & \tilde{\Omega}_{22} - \tilde{\Omega}_{21}\tilde{\Omega}_a^{-1}\tilde{\Omega}_{21}^T \end{pmatrix}.$$

Let $n = \Sigma n_{ca}$ be the total number of fish in all samples. The updated estimate of Ω is then given by

$$\text{(A.4)}\quad \tilde{\Omega} = \frac{1}{n}\left[\sum_c \sum_{a\in A_c} \sum_j r^*_{caj} r^{*T}_{caj} + \sum_a k_a R_a\right]$$

where the sum over a is over all ages in the sample and $k_a = \Sigma_c n_{ca}$. Starting with $\tilde{\Omega} = I$, the algorithm consists of alternating between (A.3) and (A.4) until a convergence criterion is met. Unless some of the correlations between growth increments are very large, rapid convergence should be expected.

At convergence, the inverse of the first bracketed term in (A.3) will estimate the variance covariance matrix of $\tilde{\theta}$ based on expected information. In the general case, where not all increments on a scale are used, the iteration has the same form as (A.3), but the sums must be over all three indices, that is, over each fish, rather than just over year-classes and age at capture.

Effects of Experimental Acidification on Zooplankton Population and Community Dynamics

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We provide experimental evidence of indirect ecological changes occurring through alterations in both competitive and predatory interactions within zooplankton communities during acidification. Acidification experiments were conducted on zooplankton in 16-m^3 enclosures suspended in a Precambrian Shield lake in central Ontario in order to identify mechanisms of community change. Changes in abundance, body size, egg ratio, and lipid status in zooplankton populations reflected changes in biotic interactions during acidification to pH between 5.0 and 6.0. Abundance of small cladocerans (especially *Bosmina longirostris*) increased following acidification despite increases in abundance or dominance of their cyclopoid predators. Increased reproduction of *Bosmina*, in the absence of detectable changes in edible phytoplankton biomass, suggests that this was due to competitive release from larger cladoceran species that did not survive acidification.

Nous apportons des preuves expérimentales des modifications écologiques indirectes qui se produisent par altération des interactions de compétition et de prédation au sein des communautés zooplanctoniques pendant l'acidification. Des expériences d'acidification ont été menées sur du zooplancton dans des enclos de 16 m^3 suspendus dans un lac du Bouclier précambrien, dans le centre de l'Ontario, pour permettre de repérer les mécanismes d'évolution de la communauté. Les changements dans l'abondance, la taille corporelle, le taux de ponte et l'état lipidique des populations de zooplancton reflétaient des changements dans les interactions biotiques pendant l'acidification, le pH atteignant 5,0 à 6,0. L'abondance de petits cladocères (particulièrement *Bosmina longirostris*) a augmenté après l'acidification malgré une augmentation de l'abondance ou de la dominance de leurs prédateurs cyclopoidés. La hausse de la reproduction de *Bosmina*, en l'absence de modifications repérables dans la biomasse phytoplanctonique comestible, semble indiquer que le phénomène est dû à un allègement de la compétition par les cladocères de grande taille qui n'ont pas survécu à l'acidification.

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Plankton community changes have been documented in acidification studies for at least two decades, but the processes involved are not clearly understood. Studies relating zooplankton community composition to physicochemical and morphometric variables in acidified lakes (e.g., Medeiros et al. 1987; Pinel-Alloul et al. 1989) have shown that direct responses to pH and related physicochemical variables explain only a portion of the observed community changes. The importance of altered species interactions in modifying the effects of acidification on zooplankton community structure was first suggested in the 1970's (e.g., Sprules 1975; Roff and Kwiatkowski 1977), but causal relationships have been difficult to establish, and consequently the processes associated with community change are still poorly substantiated.

We conducted experiments to identify causes of community change by monitoring life history traits of zooplankton populations during acid additions to enclosed water columns. We expected that this approach would lead to a better understanding of population changes (and therefore of factors influencing the population) than examination of abundance data alone, which has been the basis for identifying potential mechanisms of community change in many acidification studies. Within limits defined by the life history of the species, zooplankton populations respond to changes in predation, food availability, and water chemistry with altered body size, egg production, and lipid storage. These traits were monitored during acidification experiments conducted on plankton in enclosures in Red Chalk Lake, Ont., during the summer of 1988.

Empirical observations published in the literature (e.g., Schwartz 1984 and references cited therein) allowed us to predict responses of zooplankton life history traits to changes in invertebrate predation, food availability, or hydrogen ion toxicity. Predation by invertebrates generally removes smaller individuals, thereby increasing juvenile mortality and selecting for production of large offspring; one consequence of producing larger offspring may be fewer offspring in each brood. Reduced food availability (poor quality or inadequate quantity of food resulting either from direct effects of acidification on algae or increased competition among herbivores for food) results in slow growth rates, small brood size, and infrequent reproduction; often, animals reproduce at a smaller size. Similar effects occur with increased acid toxicity. Early mortality, slow growth, and decreased reproduction usually result (e.g., Zimmer 1987). Depending on how age structure of the population is affected, mean body size may increase (if reproduction ceases or slows, leaving mainly adult individuals) or decrease (because of slow growth and early mortality). Interpretation of changes in life history traits may not always be clearcut, since

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(1) more than one process may produce similar changes and (2) pressures may conflict, e.g., where an animal experiences a simultaneous increase in both competition and predation. It is also possible that a change in one factor will lead to a change in another, e.g., predation may reduce population abundance resulting in less competition for food.

Experiments were conducted in enclosures as a compromise between laboratory experiments (which are inexpensive and easily replicated, but too small in scale to maintain lake plankton communities, resulting in loss of what Sih et al. (1985) called "multispecies indirect effects") and between-lake comparisons (which are expensive and logistically difficult). Enclosures provide an intermediate scale of study, despite some unrealistic elements such as rapid acidification and exclusion of fish predators. Previous studies involving acidification of enclosures have shown reasonably good tracking of lake conditions during experiments lasting up to several months and changes in zooplankton species composition that were similar to those observed in lakes acidified by atmospheric deposition (e.g., Marmorek 1983; Arvola et al. 1986; Havens and DeCosta 1987; Barmuta et al. 1990).

Materials and Methods

Enclosure Setup

Experiments were carried out in Red Chalk Lake (45°11′N, 78°56′W), a clearwater oligotrophic lake on the Precambrian Shield in south-central Ontario, with alkalinity (approximately 50 $\mu eq \cdot L^{-1}$) near the median value for central Ontario lakes (Dillon et al. 1987). The lake consists of two basins separated by a narrow, shallow passage. The enclosures were placed in the 43.9-ha main basin, which has a mean depth of 16.7 m and maximum depth of 38 m.

The enclosures were transparent polyethylene cylinders, 1.5 m in diameter and 9 m in length. Each was filled with approximately 16 m^3 of water pumped from the lake using a high-capacity (500 $L \cdot min^{-1}$) water pump (Honda model WB30X). Water was pumped from approximately 2–3 m depth and was filtered through 53-μm Nitex mesh to remove most zooplankton (and, incidentally, large algae). Bag walls extended 0.5 m above the water surface to exclude lake water, and the bags were closed at the bottom to prevent contact between the enclosed water and the sediments. After temperatures of the enclosed water columns equilibrated to match those of the lake (1.5 d), zooplankton were collected with repeated vertical net hauls from 15 m (53-μm mesh) and added to the bags at night to reduce light and thermal stress on the plankton. Population densities were checked the following day, and, if required, extra net hauls were made that night to adjust zooplankton population abundances in the enclosures to resemble mean abundances in the lake.

Routine sampling and acidification began 1 wk after zooplankton were added to the enclosures. The exception to this occurred when a rip in a bag during installation allowed two young-of-year yellow perch (*Perca flavescens*) to enter. The rip was discovered before sampling began, so the enclosure was emptied, repaired, and refilled. It was then used as a control when acid treatments began the following day.

Experimental Design and Acidification

Acidification treatments focussed on effects at pH between 5.0 and 6.0, since a change from simple to complex zooplankton communities typically occurs at pH between 5.0 and 5.5 (e.g., Sprules 1975; Roff and Kwiatkowski 1977; Confer et al. 1983). This is therefore a critical pH range for plankton community structure.

The experiment was conducted from July 13 (day 0) to August 11 (day 28), 1988. Six enclosures were used: two control, two acidified in the epilimnion only (treatment AE), and two acidified throughout the enclosed water column (treatment AW). Enclosures were acidified with analytical-grade sulfuric acid to a target pH of 5.0–5.5. pH was monitored in the enclosures at least twice weekly and acid was added at irregular intervals as required to keep pH near the target levels. Epilimnetic acidification (for both AE and AW) was carried out using dilute sulfuric acid poured onto the surface and stirred in with an oar. This procedure resulted in complete mixing of acid in the epilimnion in less than 24 h, but acid was unable to cross the thermocline (midpoint at 4–6 m). Because the hypolimnion was not acidified, it was potentially available to organisms as a refuge from the acidified epilimnion in treatment AE. For treatment AW, acid was delivered to the hypolimnion in a weighted Nalgene bottle containing lake water at the temperature of the target depth.

Sampling and Analytical Methods

The enclosures and two lake stations in the main basin were sampled before acidification commenced and weekly for the duration of the experiment. A Hydrolab meter (model 4000) was used for in situ monitoring of pH, temperature, and dissolved oxygen at 1-m intervals from 0 to 9 m. Secchi depth was also measured. Temperature readings were used to identify the midpoint of the thermocline, which, to simplify sampling, was used as the boundary between the epilimnion and hypolimnion.

Epilimnetic water samples for determination of alkalinity, chlorophyll concentration, and phytoplankton abundance were collected using rubber tubing, 2 cm in diameter, which collected an integrated water sample from the surface to just above the thermocline. Hypolimnetic samples were obtained by pooling two or three casts of a Van Dorn sampler at different depths within the hypolimnion.

Water samples for alkalinity titration were stored in acid-washed Nalgene bottles (no airspace) in a cooler. Samples were titrated within 24 h using 0.01 N analytical-grade sulfuric acid and a Canlab portable pH meter (model 607) with a Corning electrode (No. 476530). Inflection-point alkalinity was calculated using a BASIC program (A.P. Zimmerman, Department of Zoology, University of Toronto, Toronto, Ont.).

Unfiltered samples for chlorophyll determinations were placed in 1-L acid-washed brown glass bottles with two or three drops of 10% magnesium carbonate and stored in a cooler for processing within 24 h. The 1-L sample was filtered onto Whatman glass microfibre filters (GF/C, pore size 1.2 μm), wrapped in foil, and frozen. Chlorophyll was extracted overnight in methanol and analysed on a Bausch and Lomb spectrophotometer following Lind (1979).

Phytoplankton samples were preserved in a mixture of 2% Lugol's solution and 50% Da Fano's fixative (50 g of cobalt nitrate, 50 g of sodium chloride, 500 mL of formaldehyde in 4.5 L of distilled water: M. Legner and W.G. Sprules, unpublished data). Phytoplankton subsamples were settled overnight in modified 13-mL Utermohl columns onto gelatin-coated slides, air-dried in a dust-free box, and mounted in glycerin.

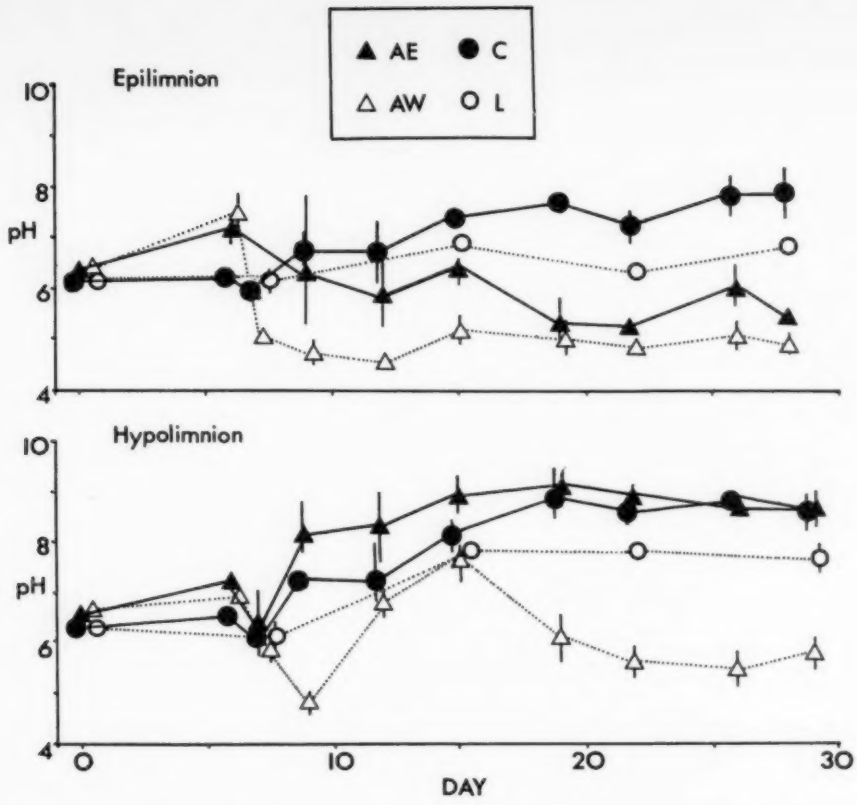

FIG. 1. Mean and range of epilimnetic and hypolimnetic pH in the treatments and lake during the experiment.

At least 200 cells or colonies from each sample were measured with an electronic caliper (Fowler Ultra-Cal II), using a compound microscope (Leitz Dialux 22) at 500× magnification. Biovolume of phytoplankton was calculated using dimensions measured for each individual and equations based on geometric solids. Biomass was calculated from biovolume, assuming a specific gravity of 1 (M. Legner, Department of Zoology, University of Toronto, Toronto, Ont., personal communication). Edible biomass in a sample was calculated as the sum of biomasses of all organisms measuring less than 30 μm in maximum dimension (Porter 1973; Richman et al. 1980).

Zooplankton were sampled separately in the epi- and hypolimnion using three vertical plankton net hauls in each stratum. A closing net, 12 cm in diameter, 1 m long, and with 53-μm mesh, was used (80-90% filtering efficiency determined by flowmeter). Fewer than 2% of the zooplankters in the enclosures were removed in each weekly sampling. The protocol for sampling zooplankton in the lake was the same as in the enclosures. All zooplankton samples were preserved in 5% sugar–formalin solution.

Zooplankton were counted and measured using a computer-based caliper system (modified from Sprules et al. 1981). Crustaceans were identified to species with the exception of copepodids and nauplii. Rotifers were identified to genus, with the exception of *Keratella taurocephala*, which is easily recognizable and considered to be an indicator of acidification (e.g., Siegfried et al. 1984; MacIsaac et al. 1987). Identification followed Edmondson (1959), Ruttner-Kolisko (1974), and Smith and Fernando (1978). Body length (maximum length minus caudal setae (copepods) or tail spines (cladocerans)), developmental stage, sex, lipid status (copepods, large cladocerans), number of eggs carried, egg stage (cladocerans), and ovary status (large cladocerans) were recorded where possible for each specimen. To assess lipid status, specimens were washed in distilled water to remove formalin and then stained with lipophilic Sudan IV (Bjorkman and Shapiro 1986). Lipid–ovary index was determined for large cladocerans following Tessier and Goulden (1982). A similar visual, qualitative assessment of lipid content (integer values from 0 (no visible lipid droplets) to 3 (many large lipid droplets)) was made for adult copepods. Biomass of zooplankton was calculated using length–weight regressions from Sprules et al. (1981).

Statistical Analysis

Repeated measures analysis of variance (ANOVA) was used to identify treatment effects on physicochemical conditions, edible and total phytoplankton biomass, zooplankton population abundances, and total zooplankton biomass. Sampling date was considered as a repeated factor because samples collected from an enclosure over time are not independent of one another. The multivariate form of the analysis as calculated by PC-SAS version 6.03 (SAS Institute, Inc. 1988) was used (N = two replicate enclosures or two lake stations), and all variables

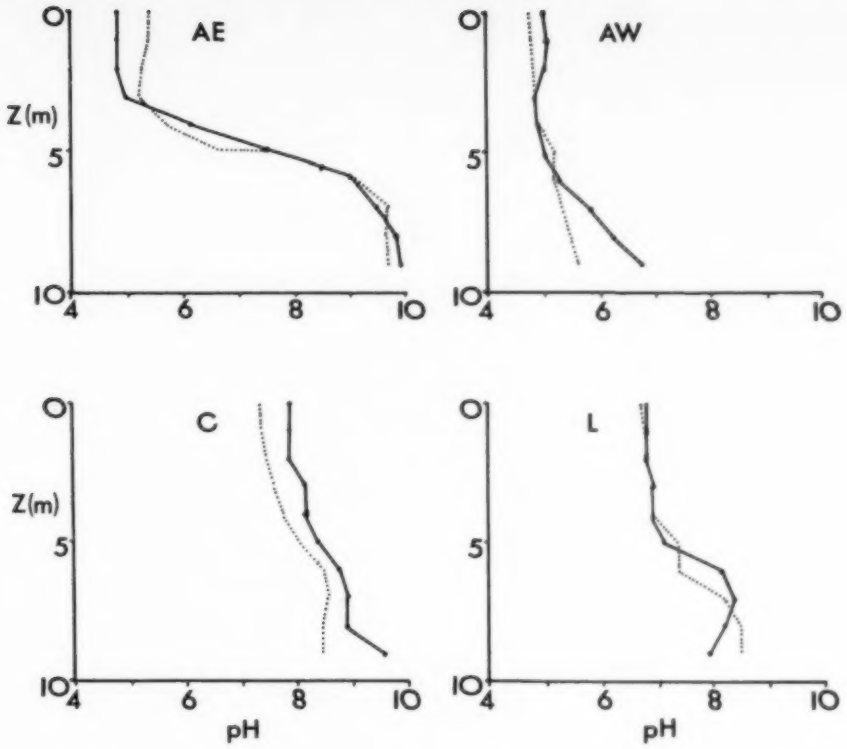

FIG. 2. Vertical pH profiles for replicate enclosures and two lake stations on August 4, 1988.

(except pH) were log-transformed. Further analysis involving single-degree-of-freedom contrasts (Sokal and Rohlf 1981; also see Hoke et al. 1990) was used to address specific questions about the pattern of responses between treatments (control (C) versus lake (L); C versus the two acid treatments (AE and AW) combined; AE versus AW). To determine when responses in the treatments changed, we examined contrasts representing the differences between adjacent time intervals.

Overall changes and replicability of zooplankton community composition were evaluated using principal components analysis with varimax rotation as calculated by PC-SAS (SAS Institute, Inc. 1988). The correlation matrix computed on logged abundances of taxa present in at least 10% of samples was analysed to examine trajectories of community composition in individual enclosures over time. Pearson's product-moment correlations of taxon abundances with principal components scores on axes 1 and 2 were used to identify associations between taxa and principal components.

Changes in zooplankton populations were assessed using measures of body size, reproduction, and condition based on log-transformed zooplankton body length and square-root-transformed egg counts, lipid index (copepods), or lipid–ovary index (large cladocerans) on days 0 and 28 of the experiment. Sizes of all females, of adult females (defined as all females larger than the smallest ovigerous female), and of ovigerous females were used as response variables determined from body length measurements. Egg count per adult female and per ovigerous female and lipid or lipid–ovary index of adult females and of ovigerous females were also used as response variables. Results from each data were analysed separately using one-way ANOVA (repeated-measures ANOVA could not be used because sample sizes were unequal (N = number of individuals)). As before, linear contrasts were used to identify effects of treatments. Specifically, effects of acidification on zooplankton abundance, egg production, body size, and lipid storage (identified by contrasts of acid and control enclosures) were compared to predicted effects of altered levels of predation, competition, and toxicity for identification of the mechanisms acting on specific populations. A similar comparison of control populations and lake zooplankton was carried out in order to identify effects related to enclosure.

Results

Significant ($P < 0.01$) overall treatment differences were observed in both epilimnetic and hypolimnetic pH (Fig. 1). Mean pH of the experimental treatments was between 5.0 and 6.0 in the epilimnion (both AE and AW), slightly higher in the hypolimnion of AW, and substantially higher in the hypolimnion of AE. Vertical profiles show general correspondence with target pH's of acidified strata, but pH increased near the bottom of bags (Fig. 2). Hypolimnetic pH was higher in AE than in any other treatment. This was apparently a consequence of hypolimnetic alkalinity generation (mechanism undetermined) following (and perhaps in response to) epilimnetic acid additions, although a similar experiment involving smaller epilimnetic acid additions (Locke 1991a) did not produce this effect. Of consequence for the experimental design is the possibility that plankton in the hypolimnion of AE may have been stressed by high pH.

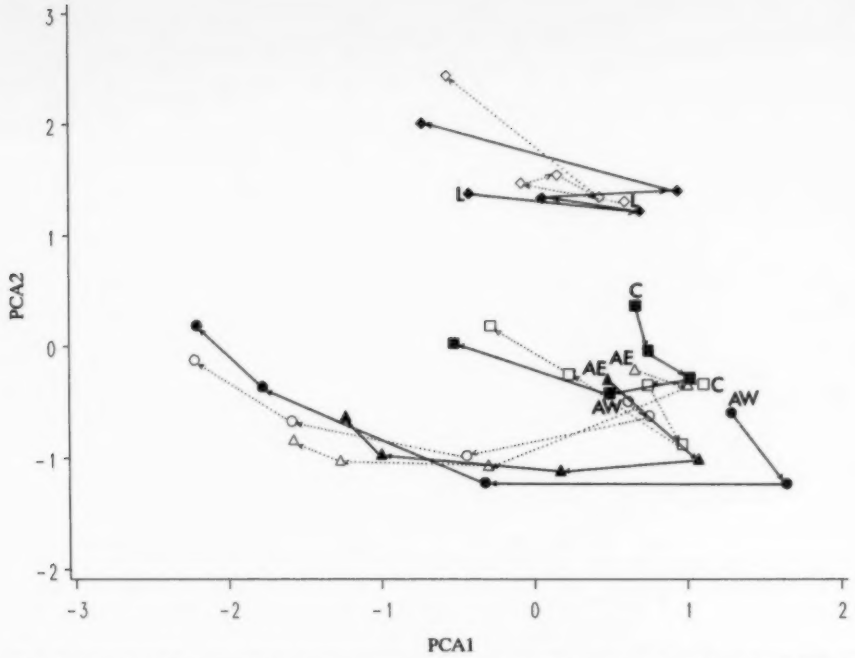

FIG. 3. Axes 1 and 2 from principal components analysis of zooplankton taxa found in at least 10% of samples. Connected points represent trajectories of individual enclosures and lake stations through time (weeks 0–4). Each trajectory is labelled with the treatment code near the starting point. Diamonds, L; squares, C; triangles, AE; circles, AW.

TABLE 1. Significant ($P < 0.05$) correlations of logged taxon abundances with the first two principal component axes.

Axis	Positive correlation	Negative correlation
PCA 1	*Asplanchna* sp.	*Euchlanis* sp.
	Conochilus sp.	*Lecane* sp.
	Keratella sp.	*Trichotria* sp.
	Daphnia galeata mendotae	*Bosmina longirostris*
	Daphnia pulex	*Chydorus* sp.
	Holopedium gibberum	
	Leptodora kindti	
	Scapholeberis kingi	
	Calanoid copepodids	
	Epischura lacustris	
	Leptodiaptomus minutus	
	Skistodiaptomus oregonensis	
PCA 2	*Asplanchna* sp.	*Chydorus* sp.
	Euchlanis sp.	*Scapholeberis kingi*
	Keratella sp.	*Sida crystallina*
	Keratella taurocephala	Copepod nauplii
	Calanoid copepodids	*Diacyclops thomasi*
	Leptodiaptomus minutus	Acari

Epilimnetic alkalinity differed among all three enclosure treatments but did not differ significantly ($P > 0.05$) between the lake and controls (Locke 1991a). Hypolimnetic alkalinity was significantly lower in AW than in other treatments, but hypolimnetic alkalinity in AE did not significantly differ from that of controls or the lake. Temperature in the enclosures closely resembled that of the lake and no oxygen depletion was observed in the enclosures during the experiment (Locke 1991a).

No significant differences were observed among enclosures or over time in chlorophyll *a*, edible and total biomass of phytoplankton, or Secchi depth (Locke 1991a). Floating filamentous algae (primarily Chlorophyta: Zygnematales) appeared in AW enclosures by day 21 but were never observed in other treatments. Plexiglas plates suspended from the enclosure frames supported a periphyton community of filamentous green algae in AW and AE and a more diverse community dominated by diatoms in controls and the lake (A. Locke, T. Howell, and L. Chisholm, unpublished data).

High replicability of zooplankton community composition was attained in each treatment (Fig. 3). Zooplankton communities from AE and AW enclosures followed a similar trajectory of change. The trajectory in control enclosures was intermediate between those in acid treatments and reference samples collected from the lake. The strongest trend in the acid treatments was a shift from the typical lake community dominated by calanoids and large cladocerans toward dominance by *Bosmina longirostris* and *Chydorus* sp. (Table 1).

Responses of species abundances and/or dominance to acid treatments (Table 2) identified taxa as (1) acid sensitive (four rotifer genera, both common *Daphnia* species, *Holopedium gibberum*, *Scapholeberis kingi*, and all three calanoid species found in the lake) or (2) acid tolerant (*B. longirostris*, *Diaphanosoma brachyurum*, *Chydorus* sp., *Sida crystallina*, cyclopoid copepods, and mites). The composition of the grazer community thus shifted from large cladocerans and calanoids to small cladocerans, with possible effects on competition for phytoplankton resources. Effects of acidification on predatory invertebrates were mixed (negative for *Epischura lacustris* but

TABLE 2. Changes in abundance and/or dominance of zooplankton following experimental acidification to pH between 5 and 6.

Taxon	Abundance	Dominance
Negative effect on abundance or dominance (− = significant effect, 0 = no effect)		
Rotifera		
Keratella sp.	−	−
Keratella taurocephala	−	0
Ptygura sp.	−	0
Euchlanis sp.	−	0
Asplanchna sp.	−	0
Cladocera		
Daphnia pulex	0	−
Daphnia galeata mendotae	0	−
Holopedium gibberum	0	−
Scapholeberis kingi	−	−
Copepoda		
Skistodiaptomus oregonensis	−	−
Leptodiaptomus minutus	−	0
Epischura lacustris	0	−
Calanoid copepodids	−	0
Positive effect on abundance or dominance (+ = significant effect, 0 = no effect)		
Cladocera		
Bosmina longirostris	+	+
Diaphanosoma brachyurum	+	+
Chydorus sphaericus	+	0
Sida crystallina	0	+
Copepoda		
Diacyclops thomasi	0	+
Mesocyclops edax	+	0
Tropocyclops prasinus	+	0
Cyclopoid copepodites	+	+
Other		
Acarina	0	+

positive for cyclopoid copepods and mites). *Chaoborus* were present in small numbers in all enclosures but were rarely collected.

The disappearance of acid-sensitive taxa from acidification treatments prevented detailed study of changes in their life history traits in response to acidification. By day 21 of the experiment, *Daphnia pulex*, *H. gibberum*, *Skistodiaptomus oregonensis*, and *E. lacustris* were no longer collected from acid treatments. By day 28, *Daphnia galeata mendotae* and *S. kingi* were also virtually eliminated.

Responses of life history traits of small cladocerans to acidification were mixed. *Bosmina longirostris* responded to acidification with decreased mean size (based on the entire population and also on adult females), but no change in the size of ovigerous females, and increased numbers of eggs/ovigerous female (although eggs/adult female decreased) (Table 3). Improvements in reproductive output of ovigerous females suggest that competition and/or toxicity was reduced. The higher proportion of small, young *Bosmina* in the population suggests that invertebrate predation in the acid treatments was not effective in controlling their abundance. *Diaphanosoma brachyurum* also responded to acidification with increased abundance of smaller sized individuals (Table 4). Since indices of reproduction and condition did not differ significantly from controls, the change in *Diaphanosoma* size was more likely due to reduction in predation than to changes in competition or toxicity. Body size of *Sida* also decreased in acidified treatments (Table 5). Reductions in eggs/adult female and lipid–ovary index suggest that increased competition or toxicity may have affected *Sida*. No significant difference was observed in any measure of size or egg production of *Chydorus* sp. (Locke 1991a).

Acid-tolerant cyclopoids were by far the most abundant predatory invertebrates in the enclosures. Feeding conditions for adult and immature cyclopoids, and survival of immature copepodids, were apparently improved in acid enclosures. Abundance of small (C1–C3) cyclopoid copepodids increased in acidified enclosures. *Diacyclops thomasi* adults and late copepodids contained increased levels of lipids (Table 6). No change was observed in size of ovigerous *Mesocyclops edax* or in the range of sizes observed, but average size decreased (Table 7), again suggesting enhanced numbers of individuals in younger copepodid stages relative to circumneutral treatments.

Discussion

This experiment demonstrates changes in zooplankton populations consistent with alterations in intensity of both (1) predation by invertebrates and (2) food limitation/competition during pH manipulations. Identification of mechanisms of community change by their effects on population life histories is sometimes ambiguous, and always subject to the constraints associated with manipulating an entire community. The major limitation is that all components of the community covary; thus, identification of causal mechanisms requires confirmation under controlled conditions, e.g., using paired-species experiments or toxicity bioassays. It is likely that the loss of many of the "acid-sensitive" species was due to toxicity, either from elevated hydrogen ion concentrations or from associated toxicants such as trace metals. *Daphnia pulex* and *D. galeata mendotae* have been shown by bioassay to be directly affected by hydrogen ion concentrations similar to those used in this experiment (Walton et al. 1982; Rago and Dorazio 1984; Havens 1990; Keller et al. 1990). *Skistodiaptomus oregonensis* and *E. lacustris* are also considered to be acid sensitive (e.g., Sprules 1975; Keller 1981), although this has not been tested by bioassays (Locke 1991b). In contrast, *Leptodiaptomus minutus* was found to be highly acid tolerant in surveys of central Ontario lakes (Sprules 1975; Keller 1981), so its poor performance in acidified enclosures was somewhat surprising. It is possible that this species responded adversely to the combined effects of acidification and enclosure, since Ramsey (1985) also found that it disappeared from acidified enclosures.

Hypotheses that could explain the increased abundance of small cladocerans include (1) reduced competition for food (either due to the loss of large cladocerans, changes in phytoplankton composition, or changes in periphyton in acid enclosures), (2) reduced predation, and (3) acidophilic response to low pH. The first two explanations are the most likely. Reduced competition associated with the disappearance of *Daphnia* is supported by studies that show that *Daphnia* outcompetes *Bosmina* at circumneutral pH (DeMott 1982; DeMott and Kerfoot 1982). The increased abundance of *Bosmina* in acid bags corresponds temporally with the loss of *Daphnia*, but also with the appearance of filamentous green algae as periphyton. Both bosminids and chydorids were probably favoured by the presence of these algae; high selectivity of *Bosmina* for filamentous

TABLE 3. Significant effects of acidification on *B. longirostris*. Results summarized here are those for which treatment effects and contrasts involving acidified enclosures were significant at $P < 0.05$ on day 28 of the experiment. Day 0 ANOVA's are provided for comparison. Significant contrasts are marked with an asterisk. Treatment codes and contrasts are further explained in the text.

		Mean (*N*) in treatment:				Significant contrasts		
Variable	Day	AE	AW	C	L	L vs. C	Acid vs. C	AE vs. AW
Size, all individuals (mm)	0	0.327	0.323	0.329	0.281	*		
		(124)	(83)	(84)	(149)			
	28	0.287	0.274	0.302	0.242	*	*	*
		(545)	(644)	(110)	(263)			
Size, adult females (mm)	0	0.328	0.328	0.338	0.308	*		
		(123)	(80)	(80)	(121)			
	28	0.305	0.299	0.329	0.276	*	*	
		(445)	(469)	(88)	(156)			
Eggs/adult female	0	0.1	0.1	0.2	0.1			
		(123)	(80)	(80)	(121)			
	28	0.1	0.1	0.3	0.2		*	
		(445)	(469)	(88)	(151)			
Eggs/ovigerous female	0	1.9	2.1	2.2	1.1	*		
		(33)	(14)	(23)	(32)			
	28	2.2	2.4	1.8	1.2	*	*	
		(106)	(112)	(37)	(57)			

TABLE 4. Significant effects of acidification on *D. brachyurum*. Results summarized here are those for which treatment effects and contrasts involving acidified enclosures were significant at $P < 0.05$ on day 28. Day 0 ANOVA's are provided for comparison. Significant contrasts are marked with an asterisk. Treatment codes and contrasts are further explained in the text.

		Mean (*N*) in treatment:				Significant contrasts		
Variable	Day	AE	AW	C	L	L vs. C	Acid vs. C	AE vs. AW
Size, all individuals (mm)	0	0.545	0.544	0.537	0.441	*	*	
		(116)	(90)	(70)	(138)			
	28	0.482	0.447	0.558	0.447	*	*	
		(125)	(91)	(77)	(131)			
Size, adult females (mm)	0	0.588	0.568	0.563	0.502	*		
		(101)	(83)	(65)	(95)			
	28	0.573	0.572	0.614	0.547	*	*	
		(80)	(47)	(63)	(69)			
Size, ovigerous females (mm)	0	0.725	0.696	0.732	0.605			
		(14)	(5)	(3)	(6)			
	28	0.604	0.596	0.685	0.583	*	*	
		(22)	(3)	(18)	(10)			

algae has been demonstrated (Fulton 1988). We have no evidence to support a change in phytoplankton composition, since our methodology would probably not have detected it, but cannot rule out this alternative. Our observations are also consistent with the second hypothesis, involving changes in the relative impact of predation on small herbivores. Absolute levels of predation were probably not reduced by acidification, since high cyclopoid abundances were maintained. Sublethal effects may have reduced predator efficiency, but this is unlikely, since high lipid levels indicate that condition of cyclopoids was good. However, levels of predation relative to prey abundance were lower in the acid treatments because of the high abundance of prey organisms, mainly *Bosmina*. The numerical response of cyclopoids to enhanced prey abundance is constrained by their reproductive seasonality. In contrast, small cladocerans respond rapidly to changes in environmental conditions and food availability, and in acid treatments were apparently able to numerically overwhelm their predators so that predation had little impact on the *Bosmina* population. The third hypothesis, that some small cladocerans are acidophilic and consequently respond positively to reduced pH, is not supported. There is no evidence from bioassay experiments that any zooplankton species are physiologically favoured by acidification (Locke 1991b). In small-scale (4 L), short-term (9-d) experiments, higher reproductive rates were observed when *Bosmina* from Red Chalk Lake were cultured in algae and water from a neutral lake than in algae and water from a lake of pH 5.8 (Locke 1991a), refuting arguments that this species is acidophilic or that it prefers the phytoplankton assemblage typical of acid lakes. Overall, the evidence suggests that competition among herbivores was reduced by removal of acid-sensitive competitively dominant species and that this was primarily responsible for the changes we observed in the zooplankton community. Changes in predation that occurred concurrently or subsequently were probably less important, since the predators alone could not have caused the observed changes in the grazer community.

For the most part the changes in zooplankton community

TABLE 5. Significant effects of acidification on *S. crystallina*. Results summarized here are those for which treatment effects and contrasts involving acidified enclosures were also significant at $P < 0.05$ on day 28 of the experiment. Day 0 ANOVA's are provided for comparison. Significant contrasts are marked with an asterisk. Treatment codes and contrasts are further explained in the text.

		Mean (*N*) in treatment:				Significant contrasts		
Variable	Day	AE	AW	C	L	L vs. C	Acid vs. C	AE vs. AW
Size, all individuals (mm)	0	1.174	1.097	1.104	1.108			
		(130)	(119)	(71)	(42)			
	28	0.959	1.048	1.319	0.871	*	*	
		(91)	(65)	(65)	(8)			
Size, adult females (mm)	0	1.664	1.726	1.877	1.639	*	*	
		(17)	(17)	(21)	(17)			
	28	1.129	1.136	1.494	0.871	*	*	
		(69)	(57)	(57)	(8)			
Eggs/adult female	0	0.1	0	0.6	0.2		*	
		(17)	(17)	(21)	(17)			
	28	< 0.1	< 0.1	0.1	0		*	
		(69)	(57)	(57)	(8)			
Lipid–ovary index	0	3.1	3.4	4.1	3.2			
		(17)	(17)	(21)	(17)			
	28	1.7	1.7	2.9	1.1	*	*	
		(69)	(57)	(57)	(8)			

TABLE 6. Significant effects of acidification on *D. thomasi*. Results summarized here are those for which treatment effects and contrasts involving acidified enclosures were significant at $P < 0.05$ on day 28. Day 0 ANOVA's are provided for comparison.

	Treatment				Significant contrasts		
Variable	AE	AW	C	L	L vs. C	Acid vs. C	AE vs. AW
Mean size (mm), day 0							
X	0.814	0.798	0.753	0.728		*	
N	129	120	116	50			
Mean size (mm), day 28							
X	0.867	0.910	0.790	0.900		*	*
N	120	82	61	3			
Lipid index, day 0							
X	2.0	1.8	1.9	1.2	*		
N	129	119	116	50			
Lipid index, day 28							
X	2.5	2.3	1.8	2.0		*	
N	120	83	62	3			

TABLE 7. Significant effects of acidification on size of adult and late copepodid stages of *M. edax*. Univariate ANOVA was used to compare transformed variates. Results summarized here are those for which treatment effect on day 28 was significant at $P < 0.05$ and contrasts involving acidified enclosures were also significant at $P < 0.05$. Day 0 ANOVA's are provided for comparison.

	Treatment				Significant contrasts		
Variable	AE	AW	C	L	L vs. C	Acid vs. C	AE vs. AW
Mean size (mm), day 0							
X	1.086	1.077	0.991	1.246		*	
N	30	26	40	39			
Mean size (mm), day 28							
X	0.896	0.941	1.072	0.863		*	*
N	40	47	34	34			

composition observed in these experiments are consistent with results of acidification surveys and whole-lake experiments, despite the potential for scale-related effects of enclosure (see Sanders (1985) for a complete discussion of enclosure effects) and the necessarily short temporal duration (at most, several generations of zooplankton). The effects we monitored represent short-term responses of the expressed levels of some life history traits to a sudden environmental shift in treatments that represent a range of conditions that zooplankton might encounter. We believe that the use of experiments at a range of spatial and temporal scales (i.e., use of enclosures coupled with smaller scale experiments to address specific hypotheses, and also com-

pared with whole-lake studies) is an effective strategy for resolving questions of community structure. Recently, the effects of spatial and temporal scale in determining the outcomes of experimental manipulations have generated considerable interest (e.g., Allen and Starr 1982; Frost et al. 1987; Wiens 1989). The experiment we conducted is a "press" experiment (Bender et al. 1984), i.e., a sustained alteration of species densities as opposed to the application of a stress after which the community is studied while it "relaxes" back to its "natural" state (a "pulse" experiment). Empirical observations suggest that for press experiments to detect bottom-up effects, they must be carried out over time periods of the order of the lifetime of the highest trophic level present (Carpenter 1987), which in our enclosures would probably mean a 1- to 2-yr experiment (i.e., the life span of copepods). The absence of significant effects on zooplankton related to phytoplankton changes in our study may therefore be an artifact of the experimental scale and does not rule out observation of these effects in lake systems. This is a question that would probably require whole-lake manipulation to answer and precludes uncritical extrapolation of our observations of transient responses to perturbations to longer term effects in larger scale systems. Failure to detect significant differences (e.g., in phytoplankton) between treatments may also result from a Type II statistical error, i.e., accepting the hypothesis that the manipulation had no effect, when in fact that hypothesis is false. Carpenter (1989) warned that use of only two or three replicates and/or modest perturbations (i.e., the conditions of our experiment) increases the risk of erroneously accepting the hypothesis of no treatment effect. Thus, treatment effects we were able to detect must have been substantial. We believe that they are not due to Type I errors because similar trends were seen in different enclosures within treatments.

In summary, our experimental results suggest that changes in zooplankton community composition are largely controlled by changes in competition resulting from removal of acid-sensitive dominant competitors. The implication is that the importance of biotic interactions such as competition and predation in determining aquatic community structure varies with pH. We cautiously apply these findings to explain similar community changes in acidified lakes.

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Effects of Ammonium and Nitrate on Growth and Domoic Acid Production by *Nitzschia pungens* in Batch Culture

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Four clones of *Nitzschia pungens* f. *multiseries* (toxin-producing form) and two of *N. pungens* f. *pungens* (non-toxic) were grown in batch culture at initial nitrogen concentrations of 55, 110, 220, 440, and 880 μM in the form of either nitrate or ammonium. As expected, only *N. pungens* f. *multiseries* clones produced the neurotoxin, domoic acid (DA). Ammonium at 880 μM prevented the growth of all *N. pungens* clones but not of *Skeletonema costatum*, although division rate was reduced. At 440 μM, ammonium lowered the cell yield obtained during the stationary phase, inhibited photosynthesis, and caused a lag phase during which DA was produced (by f. *multiseries*). At 220 and 440 μM ammonium, in contrast with the same concentrations of nitrate, stationary phase cellular DA production was enhanced by two- to fourfold. At 110 and 55 μM nitrogen, cell yield and DA production were equivalent for nitrate and ammonium but less DA was produced relative to the higher nitrogen concentrations, possibly due to nitrogen depletion. Enhanced production of DA at elevated ammonium concentrations may be a response to ammonia toxicity. This could be a useful tool for studying mechanisms of DA production and for maximizing the yield of this valuable toxin in large-scale cultures.

Quatre clones de *Nitzschia pungens* f. *multiseries* (forme toxique) et deux de *N. pungens* f. *pungens* (forme non toxique) ont été élevés en cultures discontinues avec des concentrations initiales d'azote de 55, 110, 220, 440 et 880 μM sous forme de nitrate ou d'ammonium. Comme prévu, seuls les clones de *N. pungens* f. *multiseries* ont produit de l'acide domoïque, une neurotoxine. Une concentration en ammonium de 880 μM empêche toute croissance des clones de *N. pungens* mais pas celle de *Skeletonema costatum*, dont le taux de croissance a été diminué. Une concentration en ammonium de 440 μM entraîne une réduction de la densité cellulaire obtenue en phase stationnaire ainsi qu'une inhibition de la photosynthèse, et provoque une phase de latence pendant laquelle il y a production d'acide domoïque (chez f. *multiseries*). Aux concentrations en ammonium de 220 et de 440 μM, la production d'acide domoïque par cellule pendant la phase stationnaire a été de deux à quatre fois supérieure par rapport aux mêmes concentrations en nitrate. Aux concentrations de 55 et de 110 μM, le rendement cellulaire et la production d'acide domoïque ont été équivalents pour le nitrate et l'ammonium, mais moins d'acide domoïque a été produit comparativement aux plus hautes concentrations d'azote, signe possible d'une insuffisance de cet élément. L'augmentation de la production d'acide domoïque aux hautes concentrations d'ammonium est peut-être une réaction à la toxicité de l'ammonique. Cela pourrait s'avérer utile d'une par dans l'étude des mécanismes de biosynthèse de l'acide domoïque, et d'autre part pour maximiser la production à grande échelle de cette toxine.

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In December 1987, an outbreak of food poisoning due to the consumption of blue mussels (*Mytilus edulis*) from Prince Edward Island (P.E.I.), Canada, led to the identification of a novel marine phycotoxin, domoic acid (DA) (Wright et al. 1989), and the demonstration that the toxin was produced by the pennate diatom *Nitzschia pungens* forma *multiseries* (Bates et al. 1989). The continuing problem of DA toxicity and *N. pungens* in P.E.I. during the autumns of 1988–91 allowed the study of the diatom's population dynamics and the factors influencing its growth and DA production in the field (Smith et al. 1990a). This problem now has worldwide implications because DA has appeared on the west coast of the United States (Buck et al. 1992; Fritz et al. 1992; R. Horner, University of Washington, Seattle, Wash., personal communication), and DA-producing *N. pungens* f. *multiseries* has been isolated from coastal waters of Texas (Fryxell et al. 1990) and Massachusetts (Villareal et al. 1993).

Recent laboratory investigations have shown that external nitrogen is required for DA production by nitrate-grown *N. pungens* f. *multiseries* in silicate-limited batch cultures (Bates et al. 1991). Blooms of *N. pungens* in the field are closely associated with pulses of nitrate (Smith et al. 1990a). Nitrate and other inorganic nitrogen sources probably play an important role in controlling growth and DA production by *N. pungens* f. *multiseries* in both the field and the laboratory. Ammonium is another form of combined nitrogen that is commonly available in coastal environments, especially in the presence of ammoniotelic marine animals (Dame et al. 1991). The growth of *N. pungens* on ammonium has not yet been studied. It is necessary to know which nitrogenous nutrient conditions favor *N. pungens* growth and DA production to better understand its cell physiology and to produce reliable supplies of *N. pungens* of known toxin content for studies of uptake and depuration of DA by shellfish and for toxicity experiments using zooplankton or larval fish.

The goal of this laboratory study was to compare the ability of four clones of *N. pungens* f. *multiseries* to grow and to produce DA using ammonium compared with nitrate as the nitro-

gen source. Two clones of the nontoxic *N. pungens* f. *pungens* (Smith et al. 1990b) and one of *Skeletonema costatum* from P.E.I. were included in the comparison. Several of the batch cultures contained elevated concentrations of nitrogen and therefore information on ammonium toxicity was also obtained.

Materials and Methods

The diatom clones and their origins are shown in Table 1. *Nitzschia pungens* f. *multiseries* is the DA-producing form (Bates et al. 1989), and *N. pungens* f. *pungens* is the form with no confirmed DA production (Smith et al. 1990b). The response of *N. pungens* to nitrate and ammonium was compared with that of *S. costatum*, a centric diatom also found in P.E.I. embayments. The taxonomic status of the two forms of *N. pungens* was confirmed by scanning electron microscopy (Fryxell et al. 1990; Smith et al. 1990b). The unialgal cultures were nonaxenic; both axenic and nonaxenic cultures produce DA but not at the same rate (Douglas and Bates 1992; Douglas et al. 1993). Cultures were maintained and experiments were carried out in medium f/2 (Guillard and Ryther 1962) made with filtered seawater. Stock cultures for the first ammonium experiment were maintained on 880 μM nitrate, the nitrogen concentration found in medium f/2. For subsequent ammonium experiments, cells were conditioned on 220 μM ammonium for at least 7 wk, during which the cultures were serially transferred into fresh medium a minimum of three times. Late exponential phase cultures were used to inoculate each experiment. This ensured that no DA was present in the inoculum and that the nutrient and irradiance histories of the cultures were similar. The initial concentrations of nitrogen, calculated based on dilution, were 55, 110, 220, 440, and 880 μM, provided in the form of nitrate or ammonium, as indicated. Ammonium, as ammonium chloride, was added aseptically after the f/2 medium was autoclaved. The growth temperature was 17°C and the photon flux density, provided by a bank of "cool-white" fluorescent bulbs (10 h light : 14 h dark cycle), was approximately 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ (Li-Cor, model LI-189; 4π sensor), a level sufficient to saturate growth.

Experiments were carried out (triplicate culture vessels) in either screw-capped test tubes containing 20 mL of medium, Fernbach flasks containing 1.5 L of medium, or Erlenmeyer flasks containing 250 mL of medium, as indicated. Cultures were agitated daily, and the position of the culture vessels was randomized to achieve even illumination. For experiments carried out in test tubes, growth was monitored as the change in optical density as measured with a spectrophotometer (Spectronic 20; Bausch & Lomb) at 800 nm. Cell concentration was then computed from the regression of optical density versus cell number, determined separately for each clone of each diatom species. Cell concentrations for experiments carried out in flasks were measured in triplicate by microscope counts of 5-μL culture aliquots. Cell yields (cells per millilitre) were computed by averaging at least five data points taken during the stationary phase. Chlorophyll *a* (Chl *a*) from cells collected on a glass-fibre filter (Whatman GF/F) was extracted in 90% acetone for 24 h in darkness and then measured fluorometrically (Perkin-Elmer LS-3 fluorescence spectrometer); pure Chl *a* (Sigma Chemical Co., St. Louis, Mo.) was used as a standard. The rate of photosynthesis as a function of incident irradiance was determined using a photosynthetron (Lewis and Smith 1983). Samples (1.0 mL) were incubated for 30 min with approximately 20 kBq ^{14}C-labelled bicarbonate$\cdot mL^{-1}$ (New England Nuclear Corp.), after which 50 μL of concentrated hydrochloric acid was added and the scintillation vials placed on a shaker table overnight to remove inorganic ^{14}C. Radioactivity was determined with a scintillation spectrometer (Rack Beta, model 1410; Pharmacia/LKB). The maximum rate of photosynthesis at light saturation (P_{max}) was calculated using the photosynthesis–irradiance model of Platt et al. (1980), fitted with the program "FitAll" (MTR Software, Toronto, Ont.).

TABLE 1. Diatom clones used in this study.

Species	Clone	Origin
Nitzschia pungens f. *multiseries*	NPARL	Cardigan Bay, P.E.I. (Bates et al. 1989)
	POM	Pomquet Harbour, N.S. (Seguel 1991)
	MD-1	Galveston Channel, Tex. (Fryxell et al. 1990)
	TKA-2	Galveston Channel, Tex. (Fryxell et al. 1990)
Nitzschia pungens f. *pungens*	BRUD-B	Brudenell River, P.E.I. (C. Léger, Gulf Fisheries Centre, Moncton, N.B., August 1989)
	BRUD-C	Brudenell River, P.E.I. (C. Léger, Gulf Fisheries Centre, Moncton, N.B., August 1989)
Skeletonema costatum	SCOST	Brudenell River, P.E.I. (C. Léger, Gulf Fisheries Centre, Moncton, N.B., August 1989)

DA samples were prepared by sonicating 15 mL of "whole culture" (i.e., cells plus medium) for 45 s at 210 W using a Vibra-Cell high-intensity ultrasonic processor (model VC-300; Sonic & Materials, Inc., Danbury, Conn.), followed by filtration through a disposable polycarbonate syringe filter (0.45-μm pore size, 25-mm diameter, Nuclepore) to remove the debris. DA was analyzed (limit of detection = 0.05 $ng \cdot mL^{-1}$) on a high-performance liquid chromatograph (Beckman System Gold) equipped with a Shimadzu fluorescence detector, using the FMOC fluorescence derivatization technique of Pocklington et al. (1990). Cellular DA from the "whole culture" (picograms per cell) was calculated by attributing the total DA produced in the culture to the cells.

Results

Cell Yield

The effects of three initial concentrations of nitrate (Fig. 1A) and ammonium (Fig. 1B) on cell growth are shown for *N. pungens* f. *multiseries* (clone NPARL) in batch culture. Similar results were found for *N. pungens* clones TKA-2, MD-1, BRUD-B, and BRUD-C (Table 2). None of the *N. pungens* clones grew when nitrogen was supplied in the form of ammonium at 880 μM (i.e., the nitrate-N concentration found in medium f/2). Consequently, this concentration was not used in subsequent experiments. At 440 μM ammonium, a lag phase was observed and the stationary phase cell yield was significantly lower ($P \leq 0.05$; paired Student's *t*-test) than

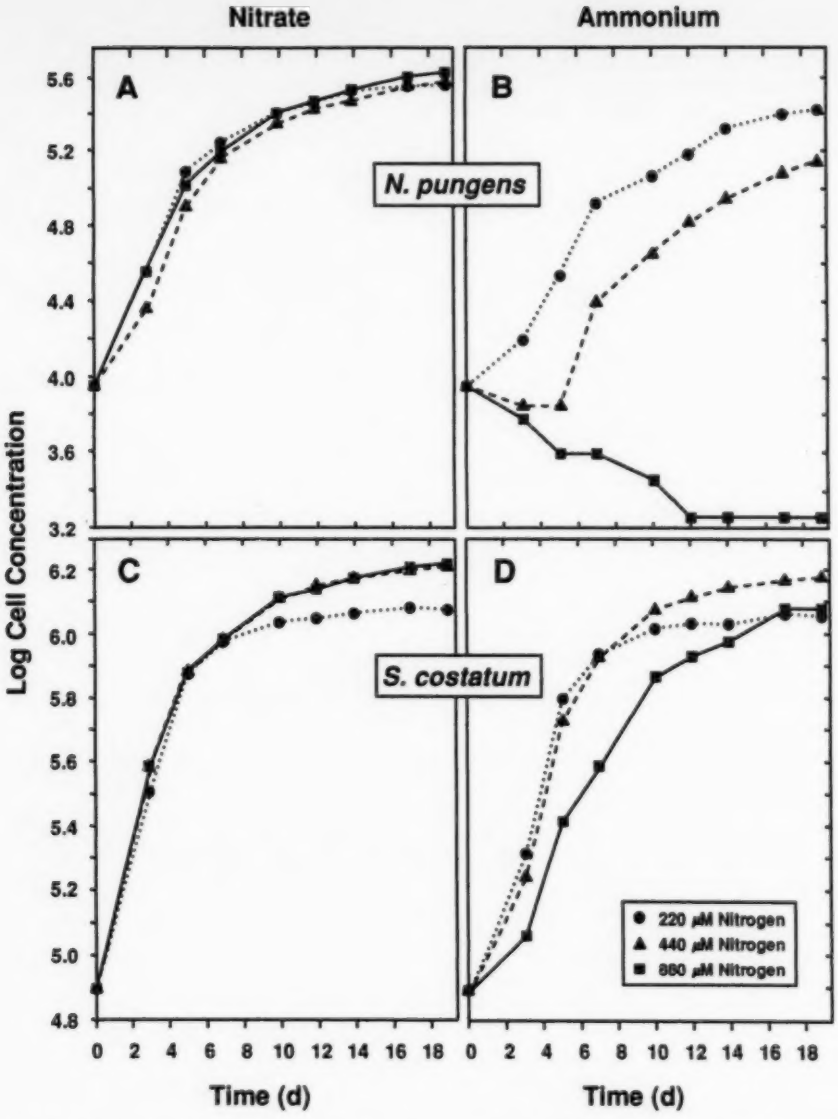

FIG. 1. Growth of (A and B) *N. pungens* f. *multiseries* (clone NPARL) and (C and D) *S. costatum* (clone SCOST) in the presence of (A and C) nitrate or (B and D) ammonium at initial nitrogen concentrations of 880 μM (squares), 440 μM (triangles), and 220 μM (circles). Values are means of triplicate cultures.

that achieved with the same nitrogen concentration in the form of nitrate. At 220 μM, ammonium still resulted in a significantly lower ($P \leq 0.05$) cell yield when comparaed with the equivalent nitrate-grown culture. By comparison, there was no significant difference ($P \leq 0.05$) in cell yield due to nitrogen concentration among the three nitrate-grown *N. pungens* cultures and no indication of growth inhibition at the highest nitrate concentration used (Fig. 1A). Compared with the results for *N. pungens*, *S. costatum* showed little difference in cell yields for growth with nitrate (Fig. 1C) or ammonium (Fig. 1D), although the division rate was lower with 880 μM ammonium.

Repeating the experiments using ammonium-grown cells preconditioned with 220 μM ammonium rather than 880 μM nitrate did not alter the growth patterns shown above. The growth curves for all six *N. pungens* clones showed similar patterns for each nitrate and ammonium concentration tested. For this reason, clone NPARL is considered representative. Mean cell yields, total DA in the culture, and DA per cell on day 18 for clone NPARL are plotted in Fig. 2 for each initial nitrogen concentration used in the form of nitrate and ammonium. Cell yields were about 25% lower in the 220 and 440 μM ammonium cultures than in the corresponding nitrate cultures, and a lower cell yield was found with 440 than with

TABLE 2. Mean cell yield (cells·mL^{-1}; thousands) during the stationary phase for *N. pungens* f. *multiseries* and *N. pungens* f. *pungens* grown with 880, 440, or 220 μM nitrogen in the form of nitrate or ammonium. Values are means (± SD) of at least five data points taken during the stationary phase. Differences in cell yield between growth in equivalent concentrations of nitrate and ammonium are significant at the 0.05 level. Graphical results for *N. pungens* f. *multiseries* (clone NPARL) and *S. costatum* from this experiment are shown in Fig. 1.

	N. pungens f. *multiseries*		*N. pungens* f. *pungens*	
Nitrogen (μM)	TKA-2	MD-1	BRUD-B	BRUD-C
Nitrate				
880	195 ± 42	265 ± 48	208 ± 29	195 ± 11
440	203 ± 33	261 ± 67	206 ± 22	189 ± 25
220	166 ± 21	246 ± 47	183 ± 14	187 ± 20
Ammonium				
880	1 ± 0	1 ± 0	2 ± 0	1 ± 0
440	30 ± 3	82 ± 7	53 ± 14	25 ± 2
220	123 ± 6	178 ± 30	132 ± 25	156 ± 5

220 μM ammonium (Fig. 2A). At 110 and 55 μM nitrogen, cell yields were equivalent for nitrate and ammonium but became diminished at the lowest nitrogen concentrations, despite the form of nitrogen used.

Domoic Acid Production

Each *N. pungens* f. *multiseries* clone showed a similar pattern of DA production with increasing nitrogen concentration; f. *pungens* clones produced no DA, as expected. On day 18, the yield of DA in the "whole culture" (i.e., cells plus medium) expressed per millilitre increased with increasing nitrogen concentration but levelled off or decreased in the cultures grown with 440 μM nitrogen (Fig. 2B). The DA yield per millilitre was similar at the two lower concentrations (55 and 110 μM) of nitrate and ammonium but was higher by twofold for clone NPARL growing with 220 and 440 μM ammonium than with the same concentrations of nitrate (Fig. 2B). The concentration of DA per cell increased with an increase in the initial nitrogen concentration (Fig. 2C). DA production was two- to fourfold greater in cells grown in 440 μM ammonium than in those grown on the same concentration of nitrate. There was no evident distinction in cell size between the nitrate- and ammonium-grown cultures to account for the differences in DA per cell.

In a time-course experiment, *N. pungens* f. *multiseries* (clone NPARL) was cultured in f/2 with 440 μM nitrogen in the form of ammonium or nitrate (Fig. 3). As in the previous experiments, the cell yield was consistently lower in the ammonium-grown than in the corresponding nitrate-grown culture (Fig. 3A). In addition, a lag phase was observed in the ammonium-grown culture, during which the DA concentration increased from 4 to 22 ng·mL^{-1} (Fig. 3B), corresponding to an increase from 0.6 to 4.2 pg·cell^{-1} (Fig. 3C). A net increase in DA concentration (from 22 to 56 ng·mL^{-1}) was also observed during an interval of near-exponential growth (days 8–13) of the ammonium-grown culture (Fig. 3B). However, the rate of increase in cell number was greater than the rate of DA production, so that the DA content per cell decreased during the exponential phase (Fig. 3C). In contrast, significant levels of DA did not appear in the nitrate-grown culture until after day 11, the beginning of the stationary phase, as expected (Bates et al. 1989). The rate of DA production decreased after day 27 in the nitrate-grown culture, perhaps due to exhaustion of nitrogen. Consistent with the above experiments, DA yield per cell was two- to threefold greater in the ammonium-grown than in the nitrate-grown culture during the stationary phase. The results were essentially the same as above when the time-course experiment was repeated with clone POM growing with 440 μM ammonium or nitrate. At 110 μM nitrogen, clone POM exhibited equivalent cell yield and DA production for both nitrate and ammonium, as was also shown in Fig. 2 for clone NPARL.

Effects on Photosynthesis

Growth at an initial concentration of 440 μM ammonium in the above time-course experiment with clone NPARL severely lowered P_{max} (the maximum rate of photosynthesis at light saturation), whether expressed per litre (Fig. 4A), per cell (Fig. 4B), or per unit Chl *a* (Fig. 4C). Compared with the nitrate-grown culture, photosynthetic inhibition in the ammonium-grown culture was most evident during the lag and exponential growth phases.

Discussion

Results of this study show that nitrate and ammonium gave equivalent growth and DA production for several clones of *N. pungens* growing in batch cultures, if the initial nitrogen concentration was 110 μM or less. When supplied at initial concentrations of greater than 110 μM, ammonium, in contrast with nitrate, appeared to be toxic to *N. pungens*: a lag phase in growth was observed, photosynthetic rates were decreased, and stationary phase cell yields were reduced. Under such conditions, DA was produced during the lag phase and a two- to fourfold enhancement in DA yield was seen during the stationary phase. A meaningful comparison of division rates could not be made because of the low sampling frequency during the exponential phase, coupled with the complex lag phase found in ammonium-grown cultures. The similarity in the response of six clones of *N. pungens* to the form and concentration of nitrogen contrasts with that of *S. costatum*, which showed little difference in cell yield with nitrate or ammonium, even at 880 μM nitrogen.

The ability to increase the DA per cell by growth on high concentrations of ammonium could be very useful for growing reliable supplies of the toxic form of *N. pungens*. Such cells could then be used for studies of DA uptake and depuration by

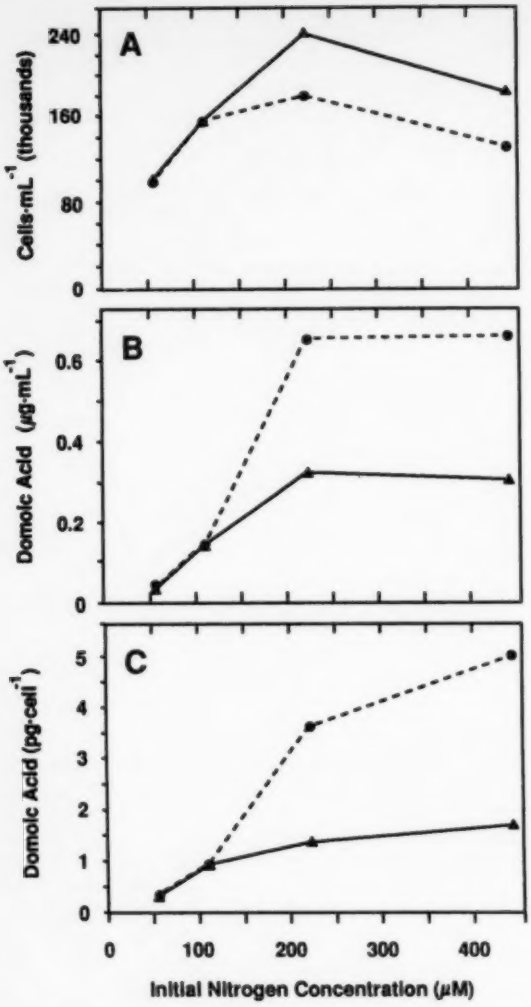

FIG. 2. (A) Mean cell yield during the stationary phase, (B) total DA in the "whole culture" (i.e., cells plus medium) on day 18, and (C) DA expressed per cell on day 18 for *N. pungens* f. *multiseries* (clone NPARL) grown at four concentrations of nitrate (triangles) or ammonium (circles).

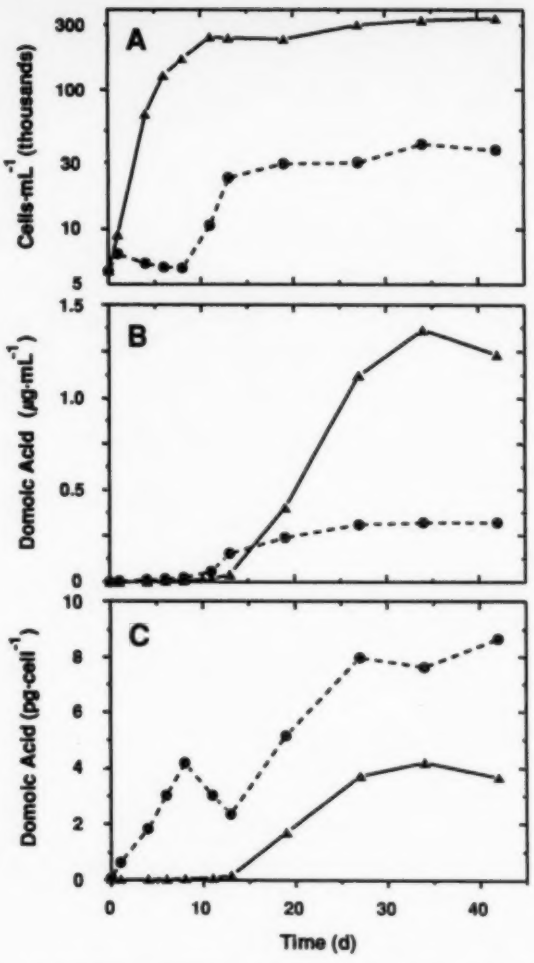

FIG. 3. (A) Growth, (B) total DA in the "whole culture", and (C) DA expressed per cell for *N. pungens* f. *multiseries* (clone NPARL) grown at an initial nitrogen concentration of 440 μM in the form of nitrate (triangles) or ammonium (circles).

shellfish and of DA toxicity to fish and invertebrates. Our results suggest that, when excreted by ammoniotelic animals under experimental conditions, ammonium may become toxic to *N. pungens* if concentrations begin to exceed 100 μM.

The toxicity of certain forms of ammonium to higher plants and marine animals is well documented (Warren 1962), although there is less information concerning phytoplankton. ZoBell (1935) found that the upper concentration limit for optimum growth of *Nitzschia closterium* (= *Cylindrotheca closterium*) was 50 μM ammonium. The growth of certain diatoms, including *Nitzschia seriata*, was inhibited by 100 μM ammonium (Guillard 1963). In contrast with our results with *N. pungens*, *S. costatum* showed less of a growth inhibition or lag period at initial concentrations of up to 880 μM ammonium (Fig. 1D). Thomas et al. (1980) similarly found no inhibition of growth or photosynthesis in *S. costatum* at 200 μM, the highest ammonium concentration they studied. As in invertebrates, fish, and mammals, toxicity to phytoplankton may involve the passage of aqueous ammonia ($NH_{3(aq)}$) through membranes (Warren 1962).

The lowered cell yields of *N. pungens* at 220 and 440 μM ammonium compared with those obtained with the same concentrations of nitrate do not appear to be related to the production of DA. This is because the same phenomenon is observed for both *N. pungens* f. *multiseries*, a proven DA producer (Bates et al. 1989), and *N. pungens* f. *pungens*, the form with no DA production (Smith et al. 1990b). It is possible, however, that f. *pungens* produces a nitrogen-containing secondary metabolite other than DA. The observed lower yields of both cells and DA at the lower concentrations (55 and 110 μM) of both nitrate and ammonium (Fig. 2) are consistent with the hypothesis that these cultures were nitrogen limited by day 18. This is supported by the finding of similar cell yields per unit nitrogen initially added for both forms and both concentrations of nitrogen: 1.42×10^6 cells·μmol^{-1} nitrate-N or ammonium-

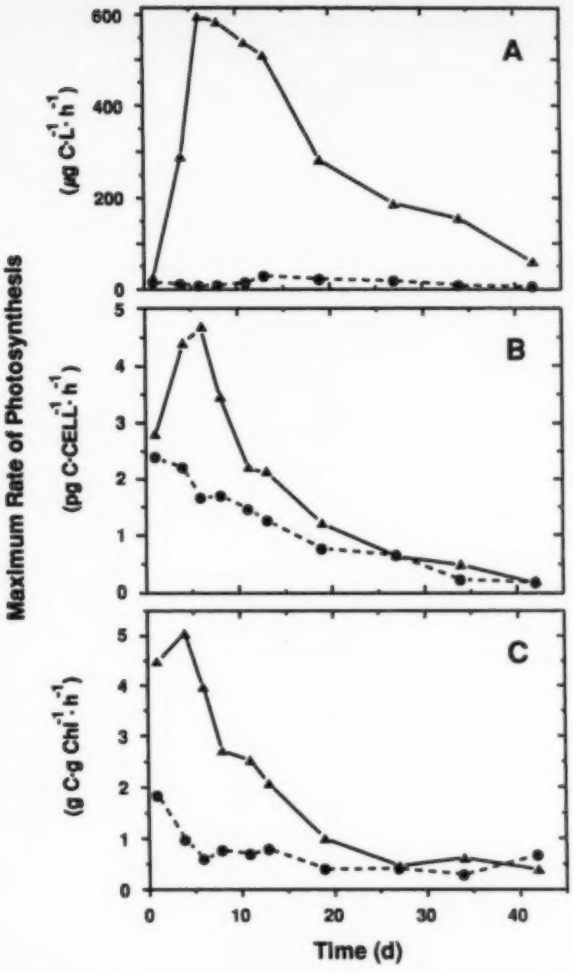

FIG. 4. Rate of photosynthesis at light saturation (P_{max}) expressed (A) per unit culture volume, (B) per unit cell, and (C) per unit Chl *a* for *N. pungens* f. *multiseries* (clone NPARL) grown at an initial nitrogen concentration of 440 μM in the form of nitrate (triangles) or ammonium (circles).

N for the 110 μM culture, and 1.85×10^6 cells·μmol^{-1} nitrate-N and 1.81×10^6 cells·μmol^{-1} ammonium-N for the 55 μM culture. The decrease in DA production rate after day 27 in the culture grown with 440 μM nitrate (Fig. 3) is also probably due to exhaustion of nitrogen (unpublished data).

As expected, only *N. pungens* f. *multiseries* clones produced DA. Previous culture experiments showed that DA was produced only after the stationary phase was reached (Bates et al. 1989, 1991). Results with nitrate as the sole nitrogen source confirm this finding (Fig. 3). The culture grown with 440 μM ammonium, however, also produced DA during the lag phase (Fig. 3C), which may have been induced by ammonia toxicity in this experiment. A factor common to both the lag and stationary phases is that the cells are not dividing. During each of these culture phases the cessation of cell division, due either to a lack of a particular nutrient (e.g., silicate; Bates et al. 1991) or to a toxic effect in an otherwise sufficient environment, is accompanied by DA production. Douglas and Bates (1992) and Douglas et al. (1993) also reported DA production during the lag phase. Thus, DA production may be an indication that the cells are not growing optimally. An exception to this, however, is that toxin production decreases under nitrogen deficiency (Bates et al. 1991), as also suggested in Fig. 2. Experiments are under way to study DA production in silicate- and phosphate-limited chemostats.

There was no difference ($P \leq 0.05$) in DA production between cultures grown with low concentrations of nitrogen (i.e., 55 or 110 μM) in the form of ammonium or nitrate (Fig. 2). This is consistent with the hypothesis that the enhanced production of DA in our experiments only occurs at initially high, potentially toxic levels of ammonium. This was observed for ammonium concentrations at or exceeding 220 μM. It is possible that ammonium toxicity may redirect some intracellular glutamate from the pathway of "normal" amino acid synthesis to that of DA synthesis (cf. Laycock et al. 1989; Douglas et al. 1992), perhaps as a response to detoxify intracellular ammonium. The increased production of DA at high concentrations of ammonium may thus be a useful tool for studying the biosynthesis of this toxin.

It is not possible to extrapolate results of this laboratory study to a field situation because of differences in the concentrations of both nitrogen and *N. pungens* cells and in the frequency and rates of supply of nitrogen. Coastal environments such as Cardigan Bay, P.E.I., nevertheless have an abundance of ammonium from such sources as agricultural runoff, remineralization, and excretion from wild and cultivated bivalves. We have started chemostat experiments with ecologically relevant supply rates of nitrogen to provide insight into the relationship between nitrogen concentration, division rate, and DA production. In addition, phytoplankton in the field are exposed to nitrate, ammonium, urea, and other organic nitrogen compounds and any of these could be used for growth (Guillard 1963). Our laboratory is also studying the effect of mixtures of these nitrogen sources on DA production.

NOTE ADDED IN PROOF: The Section Pseudonitzschia was recently removed from *Nitzschia* and was promoted to generic status (Hasle 1993). The new nomenclature for *Nitzschia pungens* is *Pseudonitzschia pungens*.

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Age- and Density-Dependent Bathymetric Pattern of Atlantic Cod (*Gadus morhua*) in the Southern Gulf of St. Lawrence

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Swain, D.P. 1993. Age- and density-dependent bathymetric pattern of Atlantic cod (*Gadus morhua*) in the southern Gulf of St. Lawrence. Can. J. Fish. Aquat. Sci. 50: 1255–1264.

I examined the bathymetric pattern of Alantic cod (*Gadus morhua*) in September in the southern Gulf of St. Lawrence for age groups 3–8$^+$ over the period 1971–91 using data from annual bottom trawl surveys. Mean age of cod tended to increase with depth in all years. The age-specific bathymetric patterns underlying this relationship differed markedly between periods of low and high abundance. When abundance was high, cod densities tended to be highest at intermediate depths, and the positive correlation between age and depth reflected a tendency for density to be highest in progressively deeper water for older fish. When abundance was low, density was either unrelated to depth or highest in shallow water, and the positive correlation between age and depth reflected a tendency for older cod to be more widely distributed than younger cod (i.e., density decreased less sharply with depth for older cod). I suggest explanations for this density dependence of cod bathymetric pattern and discuss these results in relation to the factors underlying habitat selection and bathymetric segregation by age in this population.

J'ai examiné le régime bathymétrique de la morue franche (*Gadus morhua*) en septembre dans le sud du golfe du Saint-Laurent, pour les groupes d'âge 3–8$^+$ sur la période 1971–1991, à partir des données fournies par les relevés annuels au chalut de fond. L'âge moyen des morues augmentait en fonction de la profondeur pour toutes les années. Les régimes bathymétriques spécifiques à l'âge qui sous-tendent cette relation différaient de façon marquée entre les périodes de forte et de faible abondance. Quand l'abondance était forte, les densités de morues tendaient à être maximales aux profondeurs intermédiaires, et la corrélation positive entre l'âge et la profondeur reflétait une tendance pour la densité maximale à se situer dans l'eau plus profonde chez les poissons plus âgés. Quand l'abondance était faible, la densité était soit sans rapport avec la profondeur soit au maximum dans l'eau peu profonde, et la corrélation positive entre l'âge et la profondeur montrait une tendance des sujets âgés à être plus largement répartis que les jeunes (c.-à- d. que la densité baissait moins brutalement en fonction de la profondeur chez les morues âgées). Je propose des explications de cette dépendance à l'égard de la densité dans le régime bathymétrique des morues, et j'analyse ces résultats par rapport aux facteurs qui sous-tendent le choix de l'habitat et la ségrégation bathymétrique par âge dans cette population.

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Size (or age) tends to be positively correlated with depth in many demersal fishes. Tremblay and Sinclair (1985) reported that mean depth of occurrence tended to increase with age of cod in the southern Gulf of St. Lawrence. Similarly, Sinclair (1992) noted a positive correlation between age and median depth of cod on the eastern Scotian Shelf. Macpherson and Duarte (1991) found that mean fish length tended to increase with depth in most southeast Atlantic and northwest Mediterranean demersal fishes. A positive correlation between size and depth has also been reported for several deep-sea fishes (e.g., Haedrich and Rowe 1977; Polloni et al. 1979; Snelgrove and Haedrich 1985).

Bathymetric trends in demersal fish distribution may be linked to other physical factors correlated with depth (e.g., temperature, salinity, bottom type). Temperature is a key factor in the metabolism of fishes (e.g., Fry 1971), and many studies have reported relationships between temperature and fish distributions (e.g., Scott 1982; Mahon et al. 1984; Tremblay and Sinclair 1985; Nakken and Raknes 1987; Rose and Leggett 1988; Sinclair 1992). Other studies have related fish distributions to water masses defined by specfic ranges of temperature and salinity (Perry et al. 1988; Smith et al. 1991). However, it is unlikely that fish distributions are determined by physical factors alone. Biotic factors, in particular prey abundance, have also been shown to be important determinants of distribution (e.g., Rose and Leggett 1989). Productivity generally decreases with increasing depth and distance from land (e.g. Rowe 1981; Gross 1982; Mann 1982), so prey resources may generally be greater in shallower water.

Suggested explanations for a positive correlation between depth and fish size or age have involved bathymetric trends in productivity and/or temperature. Haedrich and Rowe (1977) argued that selection for mobility and metabolic efficiency should favour large size in the relatively barren deep sea. Macpherson and Duarte (1991) noted that temperature often decreases with depth and suggested that the positive size–depth relationship in demersal fishes may result from age-specific differences in temperature preferences. They suggested that younger fish occupy warmer waters, where food supply and growth rates may be greater, whereas older fish occupy colder waters, where they may benefit from lower metabolic costs and greater longevity.

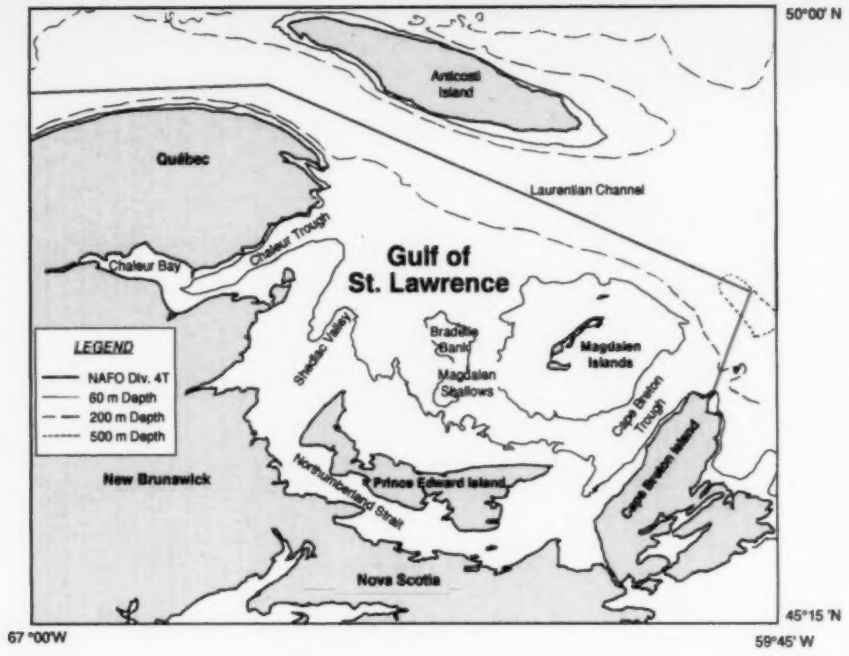

FIG. 1. Bathymetric map of the southern Gulf of St. Lawrence.

The observed bathymetric trend in mean size or age can be produced by a variety of age-specific differences in distribution. One possibility is that older fish "prefer" progressively deeper water (i.e., densities are highest in progressively deeper water for older fish). Another possibility is that fish of all ages "prefer" the same depth, but that this "preference" is stronger for older or younger fish (e.g., density may be highest in shallow water for all ages, but density may drop more sharply with increasing depth for younger fish than for older fish). These possibilities can be distinguished by examining the relationships between density and depth within age groups.

If habitats differ in levels of density-dependent resources (e.g., prey abundance, whose per capita benefit declines as population density increases), then habitat use is predicted to be density dependent (see reviews by MacCall (1990) and Rosenzweig (1991)). At low population sizes, individuals should occupy only the habitats with the highest resource levels. As population size increases, densities will increase in these habitats and their suitability will decrease due to competition, until previously less suitable habitats become equally attractive and are occupied. This predicted decline in habitat selectivity with increasing population size has been observed in a variety of animals including fishes (e.g., MacCall 1990; Rosenzweig 1991). If the depth preferences of demersal fishes are related to density-dependent resources like prey abundance, then relationships between fish density and depth should depend on population size. If depth preferences are related solely to density- independent factors like water temperature, then these relationships should be independent of population size.

In this paper, I examine annual variation in the bathymetric pattern of Alantic cod (*Gadus morhua*) in the southern Gulf of St. Lawrence. In addition to determining bathymetric trends in mean age of cod, I examine relationships between density and depth within age groups of cod. I show that age-specific relationships between cod density and depth differ between periods of low and high abundance and discuss this result in relation to the factors underlying habitat selection and bathymetric segregation by age in this population.

Material and Methods

Study Area and Population

The southern Gulf of St. Lawrence comprises a shallow shelf (mostly less than 75 m in depth) bordered on the north by the Laurentian Channel, where depth reaches a maximum of 535 m (Fig. 1). Physical oceanography of the southern Gulf is described in Strain (1988). Temperature and salinity patterns are summarized in Swain and Wade (1993). In summer, three water layers are present: a warm surface layer (10–30 m), a cold intermediate layer, and a warm deep layer (at depths greater than 150–200 m). In winter, two layers are present: a surface mixed layer with temperatures near the freezing point (−1.5°C) and the warm deep layer. Juvenile cod are protected by antifreeze proteins (Kao and Fletcher 1988) and remain in the southern Gulf throughout the year. Adult cod migrate between summer spawning and feeding grounds in the southern Gulf and overwintering grounds in the deeper, warmer water of the Sydney Bight (Jean 1964; Martin and Jean 1964).

Estimates of abundance of the southern Gulf cod stock, calculated using sequential population analysis calibrated with research vessel and commercial catch rate indices, are given in Hanson et al. (1992). Abundance was low in the early to mid-1970's and high in the early to mid-1980's (Fig. 2). The estimated midyear population size of cod aged 3 and older varied between 110 and 176 million during the period 1972–76 and between 389 and 496 million during the period 1982–87.

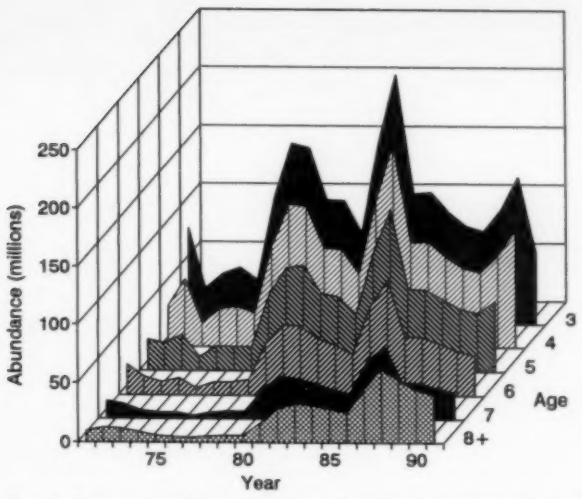

FIG. 2. Estimated midyear abundance of cod in the southern Gulf of St. Lawrence, 1971–91 (from data in Hanson et al. 1992).

TABLE 1. Number of tows and their depth distribution in annual bottom trawl surveys of the southern Gulf of St. Lawrence.

		Depth (m)		
Year	*N*	Mean	Min.	Max.
1971	66	87.5	26	265
1972	70	85.2	22	272
1973	71	83.4	20	262
1974	64	84.4	24	238
1975	67	87.9	27	274
1976	66	86.8	24	272
1977	66	90.8	26	300
1978	61	81.7	26	265
1979	74	87.9	20	351
1980	70	87.1	22	280
1981	70	86.4	20	293
1982	67	89.7	24	355
1983	67	93.4	24	333
1984	94	88.8	15	342
1985	66	94.3	22	338
1986	131	84.7	19	363
1987	130	86.3	20	365
1988	80	89.7	23	321
1989	164	80.9	23	339
1990	141	80.4	18	355
1991	186	83.7	17	369

Bathymetric Pattern

I describe the bathymetric pattern of cod in September, the time of year when cod are believed to have penetrated furthest into the Gulf and be most widely distributed (Clay 1991). Bathymetric pattern of cod was estimated for each year from 1971 to 1991 from bottom trawl surveys conducted in the southern Gulf each September. Survey procedures are described in Hurlbut and Clay (1990) and summarized in Swain and Wade (1993). Sample size ranged from 61 to 186 trawl tows per survey (Table 1). Mean depth of tows and the range of depths sampled did not vary substantially from year to year (Table 1). Cod catch at age was estimated for each tow using a two-phase stratified sampling scheme (see Swain and Wade (1993) for details). Catches were adjusted to a standard tow length of 1.75 nautical miles.

I estimated bathymetric pattern separately for fish in each age class between 3 and 7 and for fish aged 8 and older (hereafter 8^+). The relationship between cod density and depth was described for each age and year using Poisson regression models, following Smith (1990) and Smith et al. (1991). Models were of the form

$$(1) \qquad E[Y_i] = \mu_i = \exp(\beta_0 + \beta_1 X_i + \beta_2 X_i^2)$$

$$(2) \qquad \mathrm{Var}[Y_i] = \phi\, \mu_i$$

where Y_i is the number of cod of a particular age caught in tow i of a particular year, X_i is the depth of tow i, and ϕ is a parameter for extra-Poisson variation. A Poisson probability model was used because (1) variance tended to increase with the mean and (2) individual cod are sparse in relation to what the whole collection of trawl tows could contain. (When this second condition holds and individuals are randomly distributed, the number of individuals per sample unit is a Poisson variate; see Pielou (1977, p. 116).) Extra-Poisson variation was expected because organisms typically show a contagious rather than a random spatial pattern (e.g., Pielou 1977; Taylor et al. 1978). Significance of the effect of depth on cod spatial pattern was tested for each age and year by analysis of deviance (McCullagh and Nelder 1989) using the GLIM software package (Payne 1986). These tests used the change in scaled deviance between models with and without the depth term(s) being tested and are equivalent to likelihood ratio tests (McCullagh and Nelder 1989). Three tests were performed. First, the overall effect of depth was tested by removing both depth terms (X_i, X_i^2) from the full model in equation 1. Then, the X_i^2 and X_i terms were removed in sequence starting from the full model (equation 1) to test the quadratic and linear depth effects, respectively. This procedure resulted in a table of 126 probabilities (6 age groups, 21 years) for each of the effects. A sequential Bonferroni test was used to control the table-wide type I error rate (Holm 1979; Rice 1989). This procedure increases the type II error rate, a particular problem in tables of probabilities (such as those reported here) where false null hypotheses may be the rule rather than the exception. Thus, patterns in the significance of depth effects are assessed here using both the Bonferroni and uncorrected probabilities. I also calculated the proportion of the total deviance that is explained by these depth effects for each of the 126 age–year combinations. Unlike probability values, this statistic is insensitive to variation in sample size. To calculate this statistic, I divided the change in deviance due to removal of the depth term(s) by the deviance of the null model (i.e., the model containing only one parameter, representing a common μ for all the Y_i).

When the linear and quadratic terms were both significant ($P < 0.05$) for a particular age and year, the effect of depth on spatial pattern was summarized by calculating the depth of maximum predicted cod density (i.e., number per standard tow). This depth was calculated from the first derivative of the fitted model with respect to depth (i.e., $D_{\max} = -\beta_1/2\beta_2$). When the quadratic term was not significant, the effect of depth was summarized by the sign of the linear coefficient β_1.

For each age group, I tested whether bathymetric pattern was density dependent by fitting the following model to the 21-yr time series of trawl tows:

$$(3) \qquad E[Y_{ij}] = \mu_{ij} = \exp(\beta_0 + \beta_1 Z_j + \beta_2 X_i + \beta_3 X_i^2 + \beta_4 Z_j X_i + \beta_5 Z_j X_i^2)$$

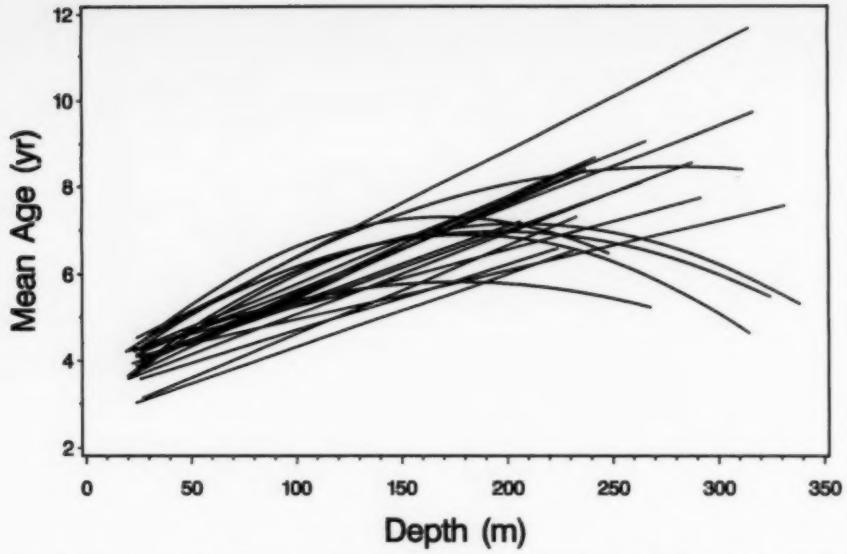

FIG. 3. Regression lines describing the relationship between mean age and depth for cod in September in the southern Gulf of St. Lawrence, 1971–91. Lines are drawn over the range of depths at which cod were caught.

(4) $\text{Var}[Y_{ij}] = \phi\, \mu_{ij}$

where Y_{ij} is the number of cod of a particular age caught in tow i in year j, Z_j is the abundance of cod of that age in year j, and X_i is the depth of tow i. The significance of the interaction between abundance and depth was tested by comparing the full model specified in equation 3 with a reduced model omitting the product terms for this interaction (i.e., $Z_j X_i$ and $Z_j X_i^2$). The change in scaled deviance between the two models was compared with a χ^2 distribution with 2 df (the difference in the number of parameters between the full and reduced models).

I tested age-specific differences in bathymetric pattern during two periods: 1972–78 and 1980–88. These two periods differed in overall abundance levels and in the form of the relationship between cod density and depth (see Results). In both cases, I used Poisson models like those described above, with a parameter for extra-Poisson variation and with year and age as factors and depth as a covariate. These models included terms for the effects of year, age, and their interaction, linear and in the second case quadratic depth terms, a term for the interaction between age and the linear depth effect, and in the second case a term for this interaction with the quadratic depth effect. The significance of differences among age classes in depth coefficients was tested by analysis of deviance comparing the full models above with reduced models omitting the term(s) for the age × depth interaction.

For comparison with results of previous workers, bathymetric trends in mean age were tested for each year by analysis of the regression of mean age per tow (ages ungrouped) on depth using the REG procedure of SAS (SAS Institute Inc. 1990).

Results

Bathymetric Trend in Age

Mean age increased ($P < 0.0004$) with depth in all 21 years (Fig. 3). A quadratic depth term was significant ($P < 0.05$) in only six years: 1972, 1986, 1987, and 1989–91. Maximum predicted mean ages were in the 170–280 m depth range in these six years. In other years, only the linear depth term was significant. The average R^2 ($\pm$ SE) for the relationship between mean age and depth was 0.43 ± 0.02, with values ranging from 0.23 (1972) to 0.64 (1988).

Age-Specific Bathymetric Pattern

Cod catch rate was related ($P < 0.05$) to depth in 106 of the 126 age–year combinations according to single-test significance levels or in 83 of the 126 cases according to a sequential Bonferroni test (Table 2). This relationship tended to be strongest during the 1980–88 period and for younger cod in years outside this period. All of the nonsignificant (single-test) cases were for older cod during the 1972–78 period (Table 2), a period of low abundance (Fig. 2).

The quadratic depth term was highly significant in most cases between 1980 and 1988 (Table 3), indicating peak catch rates of cod at intermediate depths in these years. In these years, predicted catch rates tended to be maximum at progressively greater depths for older cod (Table 4; Fig. 4). Bathymetric pattern was also less sharply peaked for older cod than for younger cod during this period (see examples in Fig. 4). These differences among age groups in bathymetric pattern were highly significant during the 1980–88 period (change in scaled deviance due to the age × depth interaction = 312.533, df = 10, $P < 0.0001$).

During the 1972–78 period, the quadratic term was significant in only four of the 42 cases according to single-test probability levels or in only one case if probabilities are adjusted to control the table-wide type I error rate (Table 3). When cod density was related to depth during this period, the maximum catch rates tended to be in shallow water for all age groups (see the 1976 example in Fig. 4). The linear depth coefficient in models without a quadratic term was negative in 39 of the 42 cases and in all of the cases with significant depth effects during

TABLE 2. Strength of the effect of depth on cod catch rate. Numbers are the percentage of the total deviance that is explained by the depth effect (both linear and quadratic terms). Asterisks indicate single-test significance levels (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$). Boldface indicates significance at the 0.05 level according to a sequential Bonferroni test.

	Age					
Year	3	4	5	6	7	8+
1971	**31.0****	**20.7*****	**19.9*****	**20.5*****	**17.9****	8.9*
1972	**19.9*****	12.3**	9.0*	7.4	5.6	6.0
1973	**29.5****	**23.5****	12.2**	10.2*	9.7*	7.2
1974	**27.7****	13.9**	7.6	7.7	5.0	3.3
1975	**44.8****	**35.4****	14.0**	7.2	5.7	3.3
1976	14.4**	10.5*	**19.5*****	15.1**	11.5*	6.3
1977	**30.2****	**19.4*****	5.7	4.3	3.8	1.5
1978	**29.6****	16.5**	4.1	0.7	0.8	0.6
1979	**36.6****	**22.2****	**16.8*****	14.0**	12.6**	9.7*
1980	**33.0****	**26.5****	**24.8****	**24.8****	**24.5****	11.6*
1981	**34.9****	**26.0****	**19.3*****	13.2**	10.3*	8.2*
1982	**18.5*****	**23.1****	**27.0****	**25.5****	**23.6****	**23.9****
1983	**28.1****	**26.5****	**22.9****	**20.7*****	**19.2*****	15.1**
1984	**21.7****	**24.7****	**29.2****	**30.9****	**30.8****	**30.9****
1985	**28.6****	**28.5****	**24.0****	**20.7*****	15.7**	11.0*
1986	**18.1****	**17.9****	**20.0****	**24.8****	**29.9****	**28.3****
1987	**26.4****	**22.4****	**28.1****	**30.3****	**31.3****	**27.7****
1988	**29.6****	**23.2****	**21.8****	**28.5****	**34.9****	**44.5****
1989	**24.4****	**15.7****	**11.2****	**10.6****	**11.9****	**15.3****
1990	**9.2*****	**8.7****	**10.4*****	**12.7****	**15.9****	**20.6****
1991	**23.6****	**12.1****	**9.4****	**10.0****	**10.1****	**9.4****

TABLE 3. Strength of the quadratic term in models relating cod catch rate to depth. Numbers are the percentage of the total deviance that is explained by the quadratic depth term. Asterisks indicate single-test significance levels (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$). Boldface indicates significance at the 0.05 level according to a sequential Bonferroni test.

	Age					
Year	3	4	5	6	7	8+
1971	11.1**	14.0***	**18.0*****	**19.7****	**17.8*****	8.7*
1972	0.2	0.4	3.3	3.3	1.1	0.1
1973	3.7	2.5	2.6	5.0	5.7*	3.4
1974	0.1	0.0	1.6	4.5	3.5	3.1
1975	5.7*	0.6	0.2	0.1	0.2	0.5
1976	1.2	0.8	2.6	1.9	1.7	0.5
1977	**15.4*****	7.9*	1.4	2.1	3.2	0.3
1978	2.5	0.3	0.1	0.5	0.4	0.6
1979	9.6**	4.1	6.5*	9.0**	11.0**	9.6**
1980	**16.4****	**15.3*****	**20.5****	**22.9****	**23.6****	11.6**
1981	**25.5****	**18.8****	12.7**	9.3**	9.1**	8.1*
1982	**16.8*****	**19.1****	**23.5****	**22.8****	**21.5****	**22.2****
1983	**18.7****	**22.1****	**19.5****	**17.6*****	**16.3*****	12.6**
1984	**12.9*****	**21.1****	**28.0****	**30.3****	**30.5****	**30.9****
1985	12.5***	**18.9****	**18.3*****	**16.7*****	13.0**	9.9**
1986	**8.7*****	**11.6****	**17.1****	2.4*	3.0*	**28.2****
1987	3.9**	**8.2*****	**23.1****	**27.8****	**30.3****	**27.4****
1988	**14.1*****	7.5**	10.1**	**24.4****	**33.1****	**44.5****
1989	0.4	0.0	1.8	5.1**	**9.3****	**14.3****
1990	0.5	3.4*	6.3**	**10.7****	**15.1****	**20.4****
1991	**6.9****	2.5*	1.5	2.8*	3.6**	4.5**

this period (Table 4; Fig. 5). These linear coefficients tended to be most significant and of greatest magnitude for the younger ages (Fig. 5). Differences among age groups in bathymetric pattern were highly significant during the 1972–78 period (change in scaled deviance due to the age × depth interaction = 86.158, df = 5, $P < 0.0001$).

The difference in the significance of the depth effect and of the quadratic depth term between the early to mid-1970's and the early to mid-1980's was not due to variation in sample size. Significance varied widely over the 1971–85 period (Tables 3 and 4) but sample size did not (Table 1). The proportion of the total deviance explained by these effects showed differences

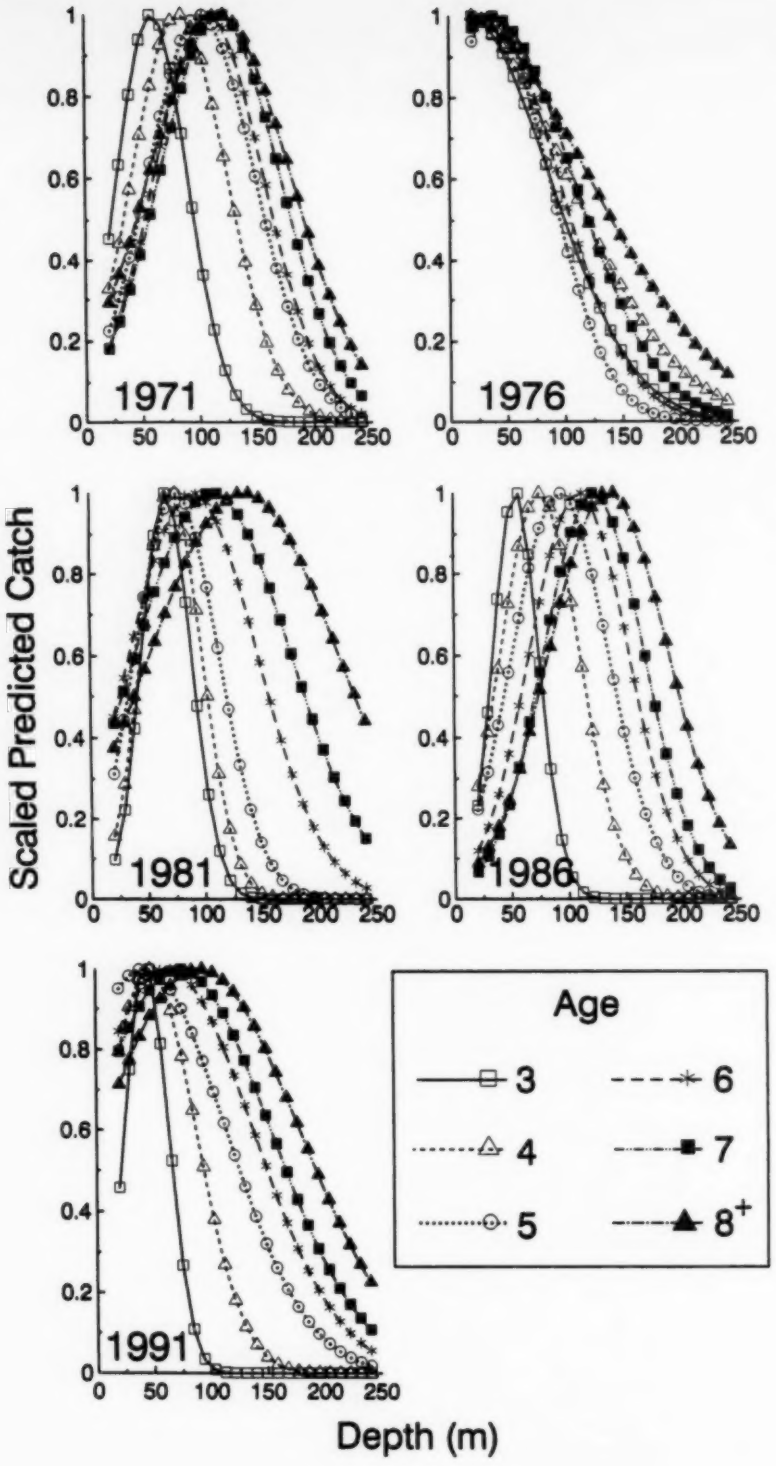

FIG. 4. Poisson regression lines describing the relationship between catch rate of cod in September and depth. Catch rates are scaled by the maximum rate for each age and year.

TABLE 4. Summary of effects of depth on cod catch rates. NS indicates no significant effect of depth ($P > 0.05$). − indicates a negative linear relationship (i.e., only the linear term is significant at $P < 0.05$). Numbers indicate maximum catch rates at intermediate depths (i.e., both linear and quadratic terms are significant at $P < 0.05$); numbers are the depth at which predicted catch rates are maximum.

	Age					
Year	3	4	5	6	7	8+
1971	58.8	82.6	101.6	108.2	117.8	117.9
1972	—	—	—	NS	NS	—
1973	—	—	—	73.4[a]	80.0	NS
1974	—	—	—	NS	NS	NS
1975	38.4	—	—	—	NS	NS
1976	—	—	—	—	—	—
1977	62.4	71.0	NS	NS	NS	NS
1978	—	—	NS	NS	NS	NS
1979	45.8	—	70.2	97.2	122.5	145.4
1980	62.2	70.9	83.9	94.0	103.9	129.3
1981	67.0	71.3	76.0	90.8	108.0	135.4
1982	93.4	83.5	82.4	85.6	94.4	105.0
1983	79.1	91.3	105.9	117.1	119.2	124.0
1984	72.6	85.9	95.9	111.1	125.7	137.4
1985	66.2	75.5	80.4	85.9	99.7	123.6
1986	54.2	75.3	93.0	109.7	122.6	135.6
1987	38.2	59.4	81.5	90.4	98.3	113.8
1988	45.1	51.8	67.5	91.4	99.0	115.7
1989	—	—	—	80.2	101.7	113.9
1990	—	72.4	89.6	110.0	124.2	132.2
1991	44.1	47.1	—	63.2	73.7	91.2

[a]Significance levels are $P = 0.02$ for the overall effect of depth, $P = 0.051$ for the linear term, and $P = 0.052$ for the quadratic term.

TABLE 5. Parameter estimates for Poisson regression models relating cod density (fish/tow) to depth (m), abundance (millions of fish), and their interaction. Models are specified by equations 3 and 4 in the text. SE = standard error of the parameter estimates. ΔS is the change in scaled deviance due to omission of the product terms for the interaction between depth and abundance (i.e., setting β_4 and β_5 to zero). P is the significance level for the interaction between depth and abundance. ϕ is the estimate of extra-Poisson variance.

	Age					
Parameter	3	4	5	6	7	8+
β_0	3.356	3.299	2.451	1.388	0.8521	0.8369
SE	0.5353	0.4219	0.3351	0.3095	0.2699	0.2564
β_1	−0.0953	−0.6441	−0.6159	0.5384	0.1442	0.7845
SE	0.4488	0.4425	0.4696	0.5323	0.7246	0.6374
$\beta_2 \times 10^2$	0.4432	−0.6555	−0.3698	1.579	1.047	1.118
SE $\times 10^2$	1.749	1.197	0.8157	0.7322	0.5740	0.4979
$\beta_3 \times 10^4$	−2.914	−0.6166	−0.0812	−0.9658	−0.5232	−0.4284
SE $\times 10^4$	1.319	0.7677	0.4428	0.3865	0.2647	0.1998
$\beta_4 \times 10$	0.1457	0.3851	0.6376	0.4450	0.9201	0.6218
SE $\times 10$	0.1376	0.1236	0.1186	0.1284	0.1578	0.1269
$\beta_5 \times 10^4$	0.1771	−1.818	−3.494	−2.022	−4.054	−2.603
SE $\times 10^4$	0.9652	0.7852	0.6815	0.7013	0.7708	0.5435
ϕ	59.24	52.43	42.69	34.73	18.62	18.23
ΔS	22.097	16.746	27.032	15.145	34.533	23.368
P	0.00002	0.0002	<0.00001	0.0005	<0.00001	<0.00001

between the two periods that are parallel to the differences in significance (Tables 2 and 3).

Density-Dependent Bathymetric Pattern

Bathymetric pattern depended on abundance for all six age groups ($P \leq 0.0005$; Table 5). For the younger age groups (3–5), predicted density was greatest in shallow water (about 20–30 m) at low abundance and at intermediate depths (70–90 m) at high abundance (Fig. 6). For the older age groups (6–8+), predicted density varied little with depth at low abundance

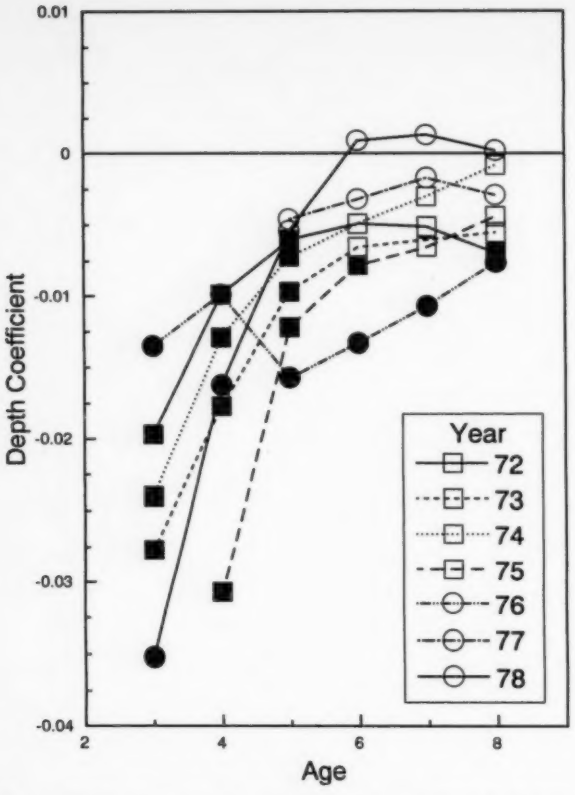

FIG. 5. Depth coefficients from models containing a linear depth term only. Coefficients are not shown for cases where the depth^2 term was significant. Solid symbols indicate coefficients significantly ($P < 0.05$) different from zero.

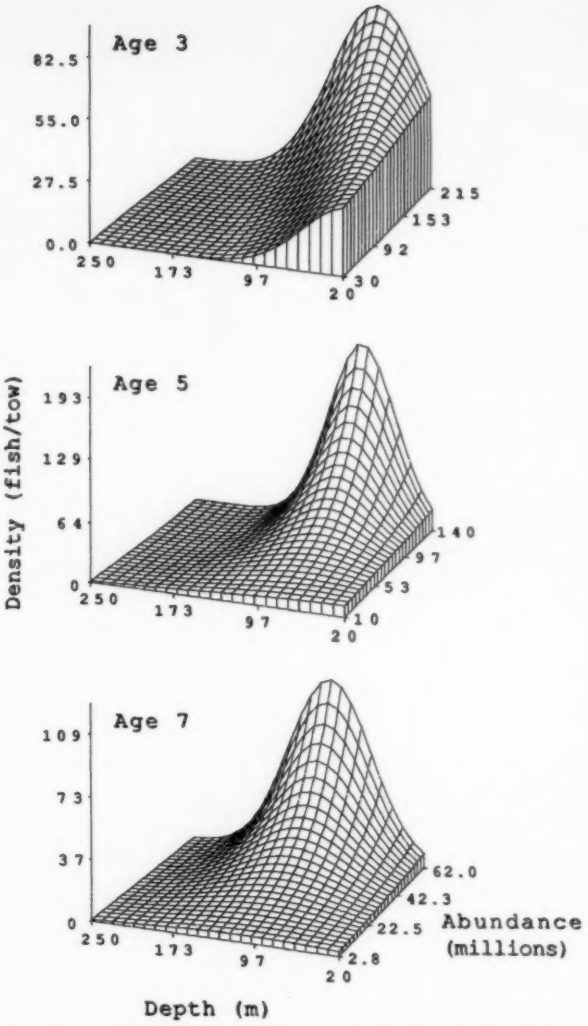

FIG. 6. Predicted relationship between cod density and depth at various levels of abundance. Predicted density is given by equation 3 in the text with parameter estimates given in Table 5.

but depended strongly on depth at high abundance (Fig. 6). Predicted density was greatest at depths of about 100 (age 6) to 120 m (age 8^+) for older cod when abundance was high.

Discussion

Mean age of cod in the southern Gulf tended to increase with depth in all years from 1971 to 1991. This result is consistent with previous findings for this population and for many other demersal fish species (Haedrich and Rowe 1977; Polloni et al. 1979; Snelgrove and Haedrich 1985; Tremblay and Sinclair 1985; Macpherson and Duarte 1991; Sinclair 1992). In the southern Gulf cod population, the age-specific bathymetric patterns underlying this relationship between age and depth differed markedly between periods of low and high abundance. During periods of high abundance, densities tended to be highest at intermediate depths, and the positive correlation between age and depth reflected a tendency for density to be highest in progressively deeper water for older fish. In contrast, during periods of low abundance, density was either unrelated to depth or highest in shallow water. In this case, the positive correlation between age and depth reflected a tendency for older cod to be more widely distributed than younger cod (i.e., the slope of the inverse relationship between catch rate and depth tended to be less steep for older cod).

The density dependence of bathymetric pattern in this study suggests that density-dependent resources are involved in the depth preferences of cod in the southern Gulf. A density-dependent resource that is likely to be an important determinant of cod distribution is prey abundance (e.g., Rose and Leggett 1989). If habitats differ in levels of such resources, then at low abundance, individuals are expected to occupy only the habitats with the highest resource levels (e.g., MacCall 1990; Rosenzweig 1991). Thus, the tendency for cod density to be greatest in shallow water when abundance is low suggests that levels of density-dependent resources are highest in shallow water.

If resource levels are highest in shallow water, why do peak catch rates of cod shift to deeper water when cod abundance is high? One possibility involves differences between habitats in effects of interference competition on foraging efficiency. Interference occurs when foraging efficiency is reduced by the

presence of competitors. If these reductions are more severe per capita in the habitat with higher resource levels, then theoretical models predict that peak density may shift from the high-resource/high-interference habitat at low population sizes to the low-resource/low- interference habitat at high population sizes (e.g., see fig. 2D in Morris 1988). Aggressive interactions are one source of interference (Fretwell and Lucas 1970) and have been observed in North Sea cod during the fall feeding season (Brawn 1961). Per capita reductions in foraging efficiency due to interference might be more severe in high-resource habitats if the more aggressive individuals occupy these habitats. Such a positive correlation between competitive ability and habitat quality is predicted to occur when there is interference (Parker and Sutherland 1986). However, aggression may not be an important source of interference at high population sizes (e.g., Gillis and Kramer 1987) and has not yet been demonstrated to affect spatial pattern at the large scales involved in this study.

A second possibility, suggested to me by D.L. Kramer, involves an interaction between effects of density-dependent resources and density-independent factors (e.g., water temperature). Suppose that density-independent costs are greater in the habitats with higher levels of density-dependent resources. In this case, peak density is predicted to occur in the high-resource/high-cost habitats at low population sizes and in the low-resource/low-cost habitats at high population sizes (D.L. Kramer, personal communication). In fishes, metabolic rate (i.e., cost) decreases as water temperature decreases (e..g, Fry 1971). Minimum water temperatures occur at depths between about 60 and 100 m in September in the southern Gulf (Fig. 7.). Thus, the shift in peak density from shallow water at low abundance to intermediate depths at high abundance may reflect a shift from high-resource (food)/high-cost (temperature) areas to low-resource/low-cost areas.

Changes in growth rate of cod are predicted as a result of these density-dependent changes in distribution. The shift in distribution from shallow water when abundance is low to intermediate depths when abundance is high involves a shift towards colder temperatures and possibly towards lower levels of prey resources. This shift suggests that competition is significant when abundance is high. Competition, colder temperature, and lower prey resources should all result in reduced growth rates at high abundance. Variation in the growth rate of cod in the southern Gulf does conform to this prediciton, with growth rate declining sharply at high population sizes (Hanson and Chouinard 1992). However, other factors (e.g., size-selective fishing mortality) may also contribute to the decline in growth rate in this population in years of high abundance (Hanson and Chouinard 1992).

Macpherson and Duarte (1991) suggested that bathymetric trends in demersal fish size may be linked to bathymetric trends in temperature. This suggestion is consistent with the second explanation proposed here for density-dependent variation in bathymetric pattern. This explanation involved a trade-off between the density-dependent benefits of greater food supplies in shallow water and the density-independent benefits of lower metabolic costs in the colder water at intermediate depths. Differences between age classes in bathymetric pattern may reflect differences in the balance between these density-dependent benefits and density-independent costs. The observed differences in bathymetric pattern suggest a number of possibilities. First, a trend toward greater prey resources in shallow water may be steeper for younger fish. This could result from size-dependent variation in diet. Diet tends to be less restricted in older individuals in many fishes (e.g., Helfman 1978), including cod in the southern Gulf (Powles 1958; Waiwood and Majkowski 1984). Second, the benefits associated with prey resources may be greater for younger fish. For example, growth may benefit younger fish more than older fish via greater reductions in size-dependent mortality with growth at smaller sizes. Greater mobility and metabolic efficiency may also permit older fish to exploit less productive habitats than younger fish (Haedrich and Rowe 1977). Third, density-dependent reductions in foraging efficiency may be greater in older fish due to stronger interference competition (e.g., limited observations suggest higher levels of aggressive interaction in older cod (Brawn 1961)). These possibilities are consistent with (1) the tendency for density to decline less sharply with depth for older cod when abundance is low and (2) the tendency for peak densities to occur at progressively greater depths for older cod when abundance is high. This explanation presumes that the second tendency reflects greater shifts in distribution for older cod along a gradient from warm (i.e., high cost) shallow water when abundance is low to cold (i.e., low cost) deep water when abundance is high. If so, when abundance is high, peak densities should be in colder water for older cod than for younger cod. Relationships between depth and temperature in the southern Gulf (Fig. 7) do not support this view. When abundance is high, peak densities occur at depths from between 40 and 90 m for age 3 cod to between 105 and 140 m for age 8^{+} cod (Table 4), while bottom temperatures appear to reach minimum values at depths near 60 m (Fig. 7). However, more direct examination

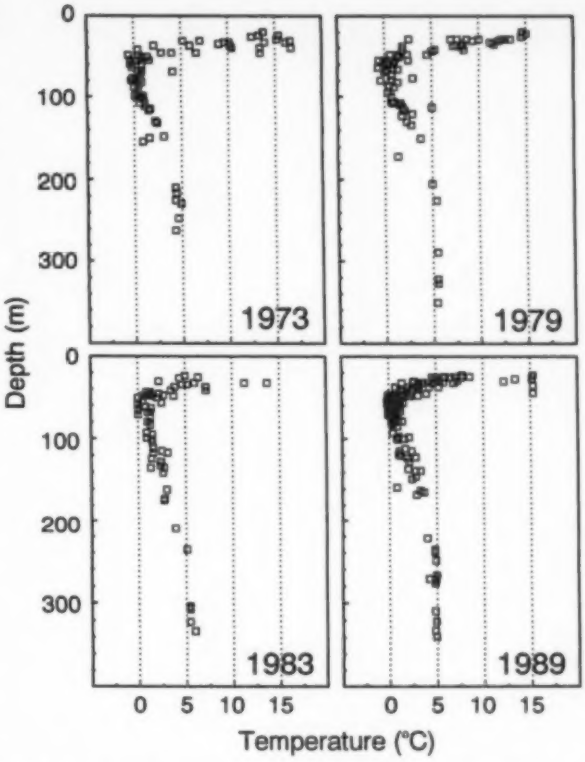

FIG. 7. Bottom temperatures recorded during research surveys in September in the southern Gulf of St. Lawrence.

of age-specific temperature distributions of cod in the southern Gulf is needed to test this prediction. This work is ongoing.

This and other studies (e.g, Sinclair 1992) demonstrate bathymetric segregation of cod by age. This means that mobile fisheries may be able to target strong year classes, with the result that the age-specific exploitation pattern of fisheries may vary annually (Sinclair 1992). The bathymetric pattern of cod in the southern Gulf of St. Lawrence was density dependent. This resulted in density-dependent variation in the extent of bathymetric segregation by age (e.g., compare 1976 and 1986 in Fig. 4) and thus presumably in the ability of fisheries to target particular year classes. A better understanding of covariation in abundance, bathymetric pattern, and age-specific exploitation patterns may improve stock assessment estimates (Sinclair 1992).

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Occurrence of Sea Lamprey, *Petromyzon marinus*, in a Newfoundland River, with Additional Records from the Northwest Atlantic

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Information on adult and larval sea lamprey, *Petromyzon marinus*, from Terra Nova River, Newfoundland, provides the first documented occurrence of a spawning population of lamprey from this province. Sea lamprey captured on the spawning grounds in mid-July 1989 ranged in length from 530 to 687 mm. Larvae collected in 1989 and 1990 ranged in length from 22 to 176 mm, and specimens were found distributed as far as 5 km below the only known spawning area. Length of larval specimens displaying signs of metamorphosis ranged from 135 to 170 mm. The length frequency distribution of sea lamprey larvae collected in 1990 is suggestive of multiple age classes. Information on the marine distribution of sea lamprey is provided from 24 records of captures in marine waters adjacent to Newfoundland.

Des données sur des adultes et des larves de grande lamproie marine, *Petromyzon marinus*, dans la rivière Terra Nova (Terre-Neuve), constituent la première étude sur la présence d'une population reproductrice de lamproies dans cette province. Les lamproies capturées sur les frayères au milieu de juillet 1989 mesuraient entre 530 et 687 mm de longueur. Les larves recueillies en 1989 et 1990 mesuraient entre 22 et 176 mm, et les spécimens étaient répartis dans une zone allant jusqu'à 5 km en aval de la seule frayère connue. La longueur des spécimens larvaires montrant des signes de métamorphose allait de 135 à 170 mm. La distribution des fréquences de longueur des larves recueillies en 1990 semble indiquer l'existence de classes d'âge multiples. L'information sur la distribution marine de la lamproie provient de 24 captures signalées dans les eaux marines proches de Terre-Neuve.

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Spawning anadromous sea lamprey, *Petromyzon marinus*, have been reported to occur in many coastal drainages from Florida north to the Bay of Chaleur (Beamish 1980). Prior to the present report, spawning populations had not been confirmed from rivers in insular Newfoundland (Scott and Crossman 1973; Beamish 1980), although there had been several anecdotal reports of sea lamprey in Terra Nova River and a confirmed sighting of a lamprey attached to an Atlantic salmon (*Salmo salar*) in Humber River, west coast Newfoundland, in the mid-1970s (L. Crane, Department of Fisheries and Oceans (DFO), Corner Brook, Nfld., personal communication). One adult specimen was captured in a fish counting fence from Sand Hill River, Labrador, in July 1971 (Murphy 1972; Anderson 1985), while a single specimen was found in Cat Arm Brook, northern Peninsula, Newfoundland (M.F. O'Connell, DFO, St. John's, Nfld., personal communication).

Reports of many sea lamprey in Terra Nova River, Newfoundland, were received by DFO personnel in the early summer of 1989. In response to these reported sightings, surveys were carried out in the section of the river where sea lamprey had been reported. This paper presents information on the first documented occurrence of spawning sea lamprey from a Newfoundland river. Additional information on the marine distribution of sea lamprey in the northwest Atlantic is provided from 24 records of marine captures complementing earlier reports (Beamish 1980; Halliday 1991).

Materials and Methods

Study Area

Terra Nova River flows into Middle Arm (Alexander Bay), Bonavista Bay, on the northeast coast of insular Newfoundland (48°40′N, 50°00′W; Fig. 1). It is a fifth-order river (Strahler 1957) with a drainage area of 1883 km^2 and has a total length of all streams of 241 km. There are waterfalls located 11.0 and 22.5 km upstream which, prior to the construction of fishways, were obstructions to upstream migrating fish. The fishway at the lower falls was constructed in 1954 and the one at the upper falls in 1952 (Porter et al. 1974). The system contains Atlantic salmon, ouananiche (landlocked Atlantic salmon), brook trout (*Salvelinus fontinalis*), Arctic char (*Salvelinus alpinus*), rainbow smelt (*Osmerus mordax*), and threespine stickleback (*Gasterosteus aculeatus*).

Data Collection

On July 17, 1989, DFO personnel from the Fisheries and Habitat Management Branch located and collected spawning sea lamprey from a section of the lower Terra Nova River called Birchy Point (Fig. 1). Several days later on July 20, we surveyed this section of the river that is located about 9 km upstream from the mouth. Walking four abreast in the stream, DFO personnel traversed an area about 150 m in length several

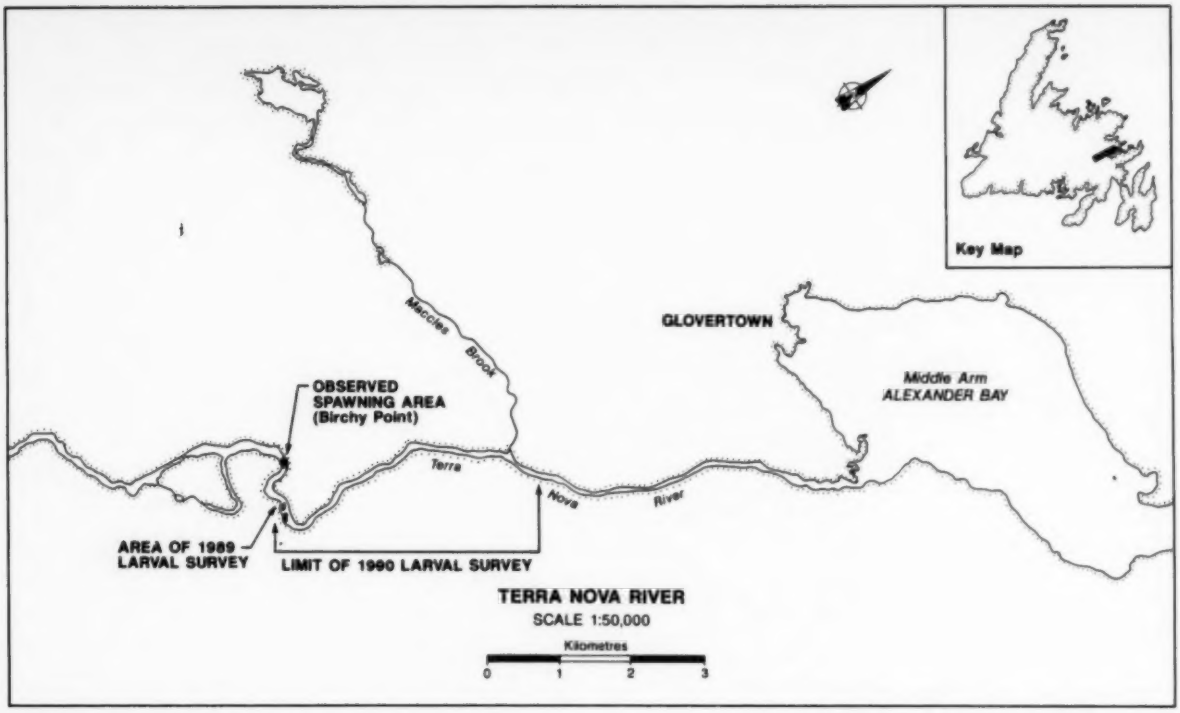

FIG. 1. Terra Nova River, Newfoundland, from the mouth to an area about 12 km upstream illustrating location of sea lamprey spawning grounds and extent of electrofishing surveys for larval lamprey.

times to observe and capture additional adult specimens. Water depth and substrate composition were also noted in areas where sea lamprey nests were observed. On both occasions, spawning sea lamprey were captured in dip nets and measured for total length (millimetres) and whole weight (grams).

Additional surveys using electrofishing equipment were carried out on September 11, 1989, and on August 28, 1990. The 1989 survey was restricted to a small area 0.5 km downstream from where the adult sea lamprey were observed to have spawned. In 1990, the spatial coverage of the electrofishing survey was extended to determine the approximate distribution of larval sea lamprey in the lower river system (Fig. 1). Six electrofishing stations, each about 75–100 m in length, were subjectively chosen from an area approximately 5 km below the site of observed spawning in 1989 (Fig. 1). At each station, electrofishing was confined to the shoreline area in water where depth was less than 1 m. Lengths and weights of larvae collected in 1990 were obtained from fresh specimens, while lengths only were recorded from larvae obtained in 1989.

Marine Catch Data Records

Information on marine distribution of sea lamprey was extracted from DFO, Newfoundland, survey records (1978–91) and from the Newfoundland Fisheries Observer Program (1977–91) (Kulka and Waldron 1983; Kulka and Firth 1987). Additional data were obtained from the personal records of the late W.J. Templeman, Northwest Atlantic Fisheries Centre, St. John's, Nfld. When available, data on date and position of capture, water temperature, capture technique, and size of specimens are provided.

Results

Spawning Sea Lamprey

In 1989, eight adult sea lamprey were collected from Terra Nova River on July 17, and five other specimens were obtained on July 20, from the Birchy Point area of the river. Sea lamprey were easily caught in dip nets and appeared to be in a weakened condition, suggestive of the final stages of their development. Specimens ranged in total length from 530 to 687 mm (mean = 601 mm) and in whole weight from 285 to 691 g (mean = 487 g). Three lamprey (23%) were females. A weight–length relationship based on the 13 specimens was

$$\log \text{weight (g)} = 2.841 \log \text{length (mm)} - 5.216,$$
$$r^2 = 0.76, P = 0.001.$$

About 100 nests were identified along the 150-m stretch of the river that had been traversed. Nests were in water depths ranging from about 25 cm to 1 m, with gravel size varying from 2 to 8 cm in diameter. Nests varied in size and shape, with the smallest ones about 30 cm in diameter and circular in shape and the larger ones upwards of 2.5 m long and 1 m wide.

Larval Lamprey

In 1989, 79 larvae were obtained from the station electrofished. Larvae ranged in length from 31 to 176 mm. No weights were recorded. In 1990, 387 larvae were captured. About 90% of the specimens captured in 1990 were from the same area we surveyed in 1989. Specimens ranged in length from 22 to 161 mm and in weight from 0.02 to 7.2 g. Densities of lamprey

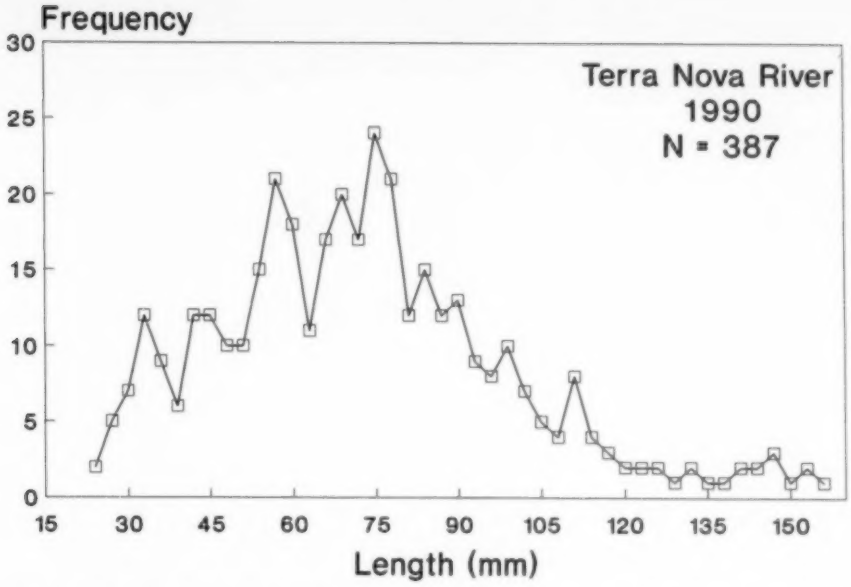

FIG. 2. Length frequency distribution of sea lamprey ammocoetes collected from Terra Nova River, Newfoundland, August 28, 1990. *N* is the number of specimens. Data are smoothed by moving averages of 7.

TABLE 1. Sea lamprey occurrences obtained from DFO, Newfoundland Region, survey records, the Newfoundland Fisheries Observer Program, and personal files of the late W.J. Templeman. Survey records include the two postmetamorphic specimens obtained by DFO personnel while sampling commercial herring catches (specimens 11 and 12). Depth and temperature data refer to average conditions for the particular set in which lamprey specimens were obtained.

					Location		Fishing details		
Source	Specimen reference No.	Length (mm)	Weight (g)	Date of capture	Latitude N	Longitude W	Depth (m)	Temperature (°C)	Gear
DFO survey	1		140	June 8, 1978	45°03′	54°33′	349	7.2	Otter trawl
	2	250	100	Feb. 14, 1981	49°02′	63°14′	381	5.4	Otter trawl
	3		300	Aug. 13, 1982	43°01′	50°36′	94	−0.2	Shrimp trawl
	4	690	600	Aug. 13, 1982	43°28′	50°35′	62	2.0	Shrimp trawl
	5		1500	Mar. 19, 1983	47°56′	48°15′	299	3.4	Otter trawl
	6	780	1500	Apr. 30, 1983	47°14′	58°35′	343	5.0	Otter trawl
	7		700	Nov. 15, 1987	43°47′	50°27′	60	1.9	Shrimp trawl
	8		240	Aug. 31, 1990	43°07′	50°37′	86	−1.1	Shrimp trawl
	9		510	Aug. 20, 1991	43°31′	51°59′	312	3.1	Otter trawl
	10		470	Sept. 7, 1991	43°32′	51°52′	140	3.4	Otter trawl
	11	146	5	Oct. 1991	48°48′	53°38′			Purse seine
	12	182	8	Oct. 1991	48°48′	53°38′			Purse seine
	13		1150	Nov. 25, 1991	48°07′	47°45′	613	3.5	Otter trawl
Observer Program	14			Feb. 14, 1979	51°51′	50°41′	415		Otter trawl
	15			July 27, 1979	44°32′	53°33′	133		Off-bottom chain
	16			Apr. 17, 1980	45°38′	58°32′	205		Otter trawl
	17			July 28, 1980	44°18′	52°55′	219		Fixed gill net
	18			Aug. 2, 1980	44°13′	52°50′	170		Fixed gill net
	19			Aug. 4, 1980	44°05′	52°51′	250		Fixed gill net
	20			Aug. 23, 1980	44°37′	52°35′	90		Fixed gill net
	21			Feb. 16, 1981	45°55′	58°30′	177		Otter trawl
	22			Nov. 26, 1987	51°13′	49°59′	700		Otter trawl
W.J. Templeman	23	677		Sept. 1, 1957	44°40′	53°42′	143		Otter trawl
	24	784	850	Feb. 1971	47°35′	48°20′	~200		Otter trawl

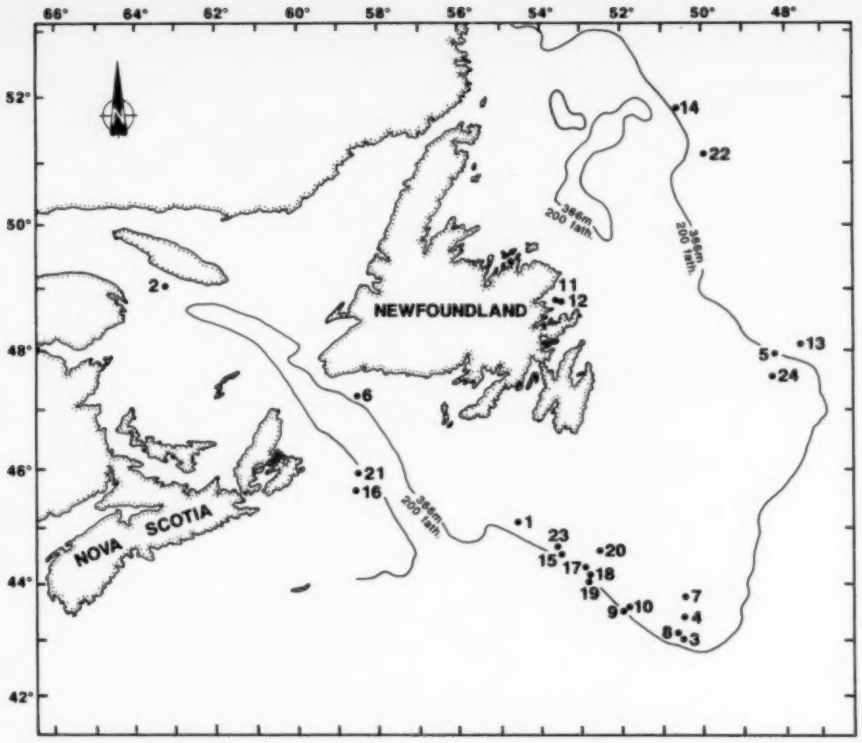

FIG. 3. Marine distribution of sea lamprey captures from DFO, Newfoundland Region, research survey records, the Newfoundland Fisheries Observer Program, and personal files of the late W.J. Templeman. Numbers and solid circles refer to specimens identified in Table 1.

decreased with increased distance downstream. The last several specimens were captured about 5 km downstream from the observed spawning area.

Specimens showing signs of initial metamorphosis (Potter 1980; Youson 1980) ranged in length from 146 to 170 mm in 1989 ($N = 6$) and from 135 to 161 mm in 1990 ($N = 3$).

The length frequency distribution of sea lamprey larvae collected in 1990, smoothed by a moving average of 7 (Hardisty 1969; Beamish 1980; Beamish and Medland 1988), is suggestive of multiple age classes (Fig. 2). No clearly defined modes, however, could be separated in larvae greater than 120 mm in size.

Marine Records

Twenty-four new marine records of sea lamprey were found in the data we examined (Table 1). Most specimens had been caught along the southwest slope and tail of the Grand Banks (Fig. 3). Depth information was available for 22 specimens, 15 of which were caught in depths less than 300 m. Specimens were caught during all seasons, and DFO survey records indicated fishing temperatures ranging from -1.1 to 7.2°C.

Length data were available for only three specimens caught during trawl surveys: 250, 690, and 780 mm for specimens 2, 4, and 6, respectively. Weights were recorded for all specimens caught in DFO surveys (Table 1). Two recently metamorphosed sea lamprey were caught in Bonavista Bay, Newfoundland (specimens 11 and 12, Fig. 3) in a purse-seine net along with a catch of Atlantic herring (*Clupea harengus*). These specimens were 146 and 182 mm in length and 4.8 and 8.0 g in weight, respectively. No size data were available from the Observer Program records.

Discussion

Results presented in this paper confirm the presence of a spawning population of sea lamprey in a Newfoundland river. Occurrence of reproducing sea lamprey in other Newfoundland rivers is unknown. Operation of fishways and fish counting fences (Moores and Ash 1984) and baseline surveys carried out in numerous watersheds throughout Newfoundland over the years have failed to note the existence of sea lamprey populations. Recreational fishing for Atlantic salmon occurs in over 150 rivers in insular Newfoundland. Peak fishing activity for salmon in Newfoundland would often coincide with the timing of sea lamprey spawning runs. Yet, apart from Terra Nova River, no observations of sea lamprey concentrations or sea lamprey carcasses have been reported.

Availability of optimal stream substrate, in particular, sufficient proportions of sand and silt habitats, has been cited as a possible factor limiting the distribution of sea lamprey within the Great Lakes (Young and Kelso 1990). This would undoubtedly be important in Newfoundland where most river systems are generally characterized by gravel, cobble, and boulder substrates (M.F. O'Connell, DFO, personal communication).

Age structure of larval lamprey populations has often been inferred from length frequency distributions (Beamish and

Medland 1988), although not without expressed caution regarding the use of this technique (Beamish and Potter 1975; Beamish 1980; Potter 1980). However, in some studies, length frequency distributions have provided comparable results with aging using statoliths (Beamish and Medland 1988). In the absence of corroborative information from statoliths, definitive conclusions pertaining to the age structure and growth of sea lamprey larvae from Terra Nova River cannot be made. At best, comparisons of the larval length frequency distribution from the Terra Nova River with distributions from other populations where confirmed aged specimens were available (Beamish and Medland 1988) suggest that multiple age classes, possibly up to five or six years are present. If correct, this implies that sea lamprey have been spawning in Terra Nova for a number of years.

Although spawning occurred in July in 1989, information is lacking on the timing and duration of the spawning migration of adults. As few other lamprey were observed during the July survey, it is possible that these specimens represented the last of the spawning component for that year. This would be consistent in relation to the timing of the initial calls received by DFO, in late June and early July, concerning sightings of sea lamprey in the river.

The two recently metamorphosed sea lamprey captured in November 1991 in Bonavista Bay, Newfoundland, were probably from Terra Nova River. The sizes of these two specimens were consistent with those larval samples from Terra Nova River that displayed early signs of metamorphosis. This suggests some evidence for an autumn migration of at least part of the population.

The occurrence of sea lamprey has been documented in the western Atlantic from latitude 36°02′ to 53°01′N (Beamish 1980; Halliday 1991). The additional marine records reported here are consistent with those of Beamish (1980) and Halliday (1991) but extend the distribution easterly from longitude 52°15′ to 47°45′W. Halliday (1991) has proposed a pelagic and wide-ranging distribution along the shelf edge and over the continental slope for larger sea lamprey. This is consistent with many of the observations reported in the present study. Many of the marine records were captured 300 km or more away from the Newfoundland coast, with the most distant record (specimen 3) caught in a shrimp trawl about 450 km from the coastline. Given the rare occurrence of sea lamprey in Newfoundland rivers, these distant incidences provide additional support for Halliday's proposal for an extensive pelagic distribution.

Most specimens reported in this paper were found in water depths of less than 300 m and are consistent with previous records (Beamish 1980; Halliday 1991). Although research vessel surveys in the Newfoundland region routinely fish at depths down to 700–1000 m (J. Baird, DFO, St. John's, Nfld., personal communication), Beamish (1980) indicated that lamprey could have been caught as the fishing gear was being set or hauled. If it is assumed that the lamprey were captured during the actual trawling process along the bottom, then one specimen reported in this paper was captured at a fishing temperature of −1.1°C. This temperature is consistent with other temperatures recorded at that time in this area (S. Walsh, DFO, St. John's, Nfld., personal communication) and would represent an occurrence of lamprey at a temperature colder than previously recorded. The conductivity, temperature, and depth (CTD) profile associated with this particular trawl set from which the lamprey was captured indicated that subzero water began at a depth of about 49 m with −1°C water below 74 m. Alternatively, the specimen could have been captured at the surface or in midwater. Surface temperature was 17°C, but dropped rapidly from 13.5°C at 13 m to 1.5°C at 30 m.

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Interactive Effects of Nutrient Availability and Light Levels on the Periphyton Composition of a Large Oligotrophic Lake

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Marks, J.C., and R.L. Lowe. 1993. Interactive effects of nutrient availability and light levels on the periphyton composition of a large oligotrophic lake. Can. J. Fish. Aquat. Sci. 50: 1270–1278.

We investigated the effects of nitrogen, phosphorus, and light on periphyton in a clear oligotrophic lake. Experimental treatments consisted of three levels each of nitrogen, phosphorus, and light in a three-way factorial design. Nitrogen and phosphorus concentrations were manipulated using nutrient-diffusing clay substrates; light levels were manipulated with shade cloth. Periphyton biovolume and cell densities increased significantly with both nitrogen and phosphorus enrichment but were not affected by shading. Phosphorus enrichment increased periphyton accumulation only when nitrogen levels were high, indicating a significant interactive effect between nitrogen and phosphorus. Differences in species composition among treatments were mostly due to changes in the diatoms *Nitzschia palea* and *Rhopalodia gibba*. Ambient and phosphorus-enriched substrates were dominated by *R. gibba*, which contains a nitrogen-fixing endosymbiont, and blue-green algae. Nitrogen enrichment and nitrogen and phosphorus in combination resulted in an assemblage dominated by *N. palea*. Unshaded and highly shaded substrates were dominated by *N. palea* and *Achnanthes minutissima*, but the medium-shade substrates were dominated by *R. gibba*.

Nous avons étudié les effets de l'azote, du phosphore et de l'éclairement sur le périphyton d'un lac oligotrophe aux eaux claires. L'expérimentation a consisté à appliquer selon un plan factoriel triangulaire trois niveaux de concentration d'azote et de phosphore et de quantité de lumière. Les concentrations d'azote et de phosphore ont été manipulées à l'aide de substrats argileux diffusant des nutriants; le niveau d'éclairement a été manipulé avec une toile à ombrer. Le biovolume du périphyton et les densités des cellules ont augmenté de façon notable du fait de l'enrichissement en azote et en phosphore, mais n'ont pas été affectés par l'ombrage. L'enrichissement en phosphore n'accroissait l'accumulation de périphyton que lorsque la concentration d'azote était élevée, ce qui indique une interaction notable entre l'azote et le phosphore. Les différences dans la composition spécifique entre les divers traitements étaient dues essentiellement à des modifications chez les diatomées *Nitzschia palea* et *Rhopalodia gibba*. Les substrats ambiants et enrichis au phosphore étaient dominés par *R. gibba*, qui contient un endosymbiote fixant l'azote, et par des algues bleues. L'enrichissement à l'azote et l'apport d'azote et de phosphore en combinaison produisaient un assemblage dominé par *N. palea*. Les substrats ombragés et fortement ombragés étaient dominés par *N. palea* et *Achnanthes minutissima*, mais les substrats moyennement ombragés étaient dominés par *R. gibba*.

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Studies of the effects of nutrients and light on attached algae have both theoretical and practical applications. Human land use developments increase organic input into watersheds, often in the form of nitrates and phosphates; water clarity may be reduced in disturbed areas due to plankton blooms following enrichment and direct input of particulate matter. Periphyton assemblages are useful for testing hypotheses concerning the effects of resource levels on species composition because they have high species diversity, are easily manipulated, and respond rapidly to altered resource levels. Manipulative studies focus on determining which resources limit periphyton growth (Gregory 1980; Fairchild and Lowe 1984; Grimm and Fisher 1986; Cattaneo 1987) and on testing predictions of Tilman's resource ratio model (Tilman 1982; Fairchild et al. 1985). Recently, Tilman (1985) has attributed more importance to light as a universally limiting resource. Evidence suggests that many terrestrial plant species are colimited by a soil nutrient and light availability. Periphyton accrual has been shown to positively correlate with light availability in lentic systems (Stevenson and Stoermer 1981; Hudon and Bourget 1983; Hoagland and Peterson 1990). Thus, extending Tilman's (1985) model to aquatic ecosystems, the interaction of nutrient concentrations and light availability could explain much of the variation seen in periphyton accumulation and species composition among habitats.

We studied the independent and interactive effects of nitrogen, phosphorus, and light on attached algae in a large oligotrophic lake. Specifically, we addressed the following questions: (1) How do nitrogen, phosphorus, and light independently influence periphyton biomass in a large oligotrophic lake? (2) How do individual populations of algae respond to changes in these parameters? (3) How do changes in these resources

affect species composition? (4) What types of interactive effects occur among these factors? There have been no studies that experimentally manipulate these three resources in a lake. This experiment was conducted in Flathead Lake, Montana, an oligotrophic lake with high light penetration. We experimentally enhanced nutrient regimes with nutrient-diffusing substrates and reduced light levels with shade cloth. Because many western North American watersheds are nitrogen limited, we predicted that nitrogen would limit periphyton growth under high ambient light. However, as nitrogen levels were experimentally increased, periphyton would become phosphorus limited and increases in phosphorus would then result in increased algal accumulation. We predicted, in contrast, that under heavy shading, periphyton would be light limited and would not respond to nutrient enrichment.

Materials and Methods

Study Site

Flathead Lake, Montana, lies at an elevation of 879 m and has a drainage basin area of 18 379 km^2. The area of the lake is 495.9 km^2 and the mean depth is 50.2 m. Human activity is currently increasing within its drainage basin (recreation, coal mining, oil drilling, logging, farming), although most of the catchment is still relatively undisturbed. Flathead Lake has been previously classified as oligotrophic (Potter and Stanford 1975); however, based on phytoplankton densities and conspicuous mats of benthic algae in the littoral zone, Stanford et al. (1983) suggested that the lake be reclassified as meso-oligotrophic. This change in trophic status may be due to accelerated anthropogenic nutrient loading (Stanford et al. 1983). Despite increases in algal densities, ambient nutrient concentrations remain low in Flathead Lake. Mean annual total phosphorus is 7.2 $\mu g \cdot L^{-1}$ and nitrate nitrogen is 69.8 $\mu g \cdot L^{-1}$. Nutrient concentrations remain fairly constant throughout the year except for during spring freshet (May 15 – June 10) at which time phosphorus concentrations are 2–10 $\mu g \cdot L^{-1}$ above the yearly mean (Stanford et al. 1983). The hypolimnion is well oxygenated throughout the year, and the Secchi disc depth is >18 m.

In situ Periphyton Experiment

Clay flowerpot dishes, with an outer diameter of 11.5 cm and an internal volume of 200 mL, were used as nutrient-diffusing substrates. Eighty-one clay substrates were constructed using a modified version of the method described by Fairchild and Lowe (1984). The dishes were acid washed and filled with specified concentrations of nitrogen and phosphorus in 2% agar. They were sealed by attaching a 12.5-cm^2 piece of 2-mm Plexiglas over the mouth with inert silicon sealant. Nitrogen and phosphorus treatments included all possible combinations of 0.00, 0.05, and 0.50 M solutions of $NaNO_3$ with 0.00, 0.05, and 0.50 M solutions of KH_2PO_4, resulting in nine nutrient treatments. Nine 60-cm^2 grids were constructed by welding rebar rods together. Hardware cloth was wired across each grid. Nine substrates (one of each nutrient treatment) were attached (Plexiglas side down) to each grid with wire. On three of the grids, a light 1.0-mm-mesh nylon screening shade was stretched across the grids approximately 15 cm above the substrates on a rebar frame; these grids were the medium-shade treatments. On three other grids, heavier shade cloth was attached to produce a highly shaded environment. The remaining three grids had no shade cloth. This design created a complete factorial combination of nine nutrient treatments × three light levels. Each treatment was replicated three times for a total of 81 substrates.

On July 7, 1988 (day 1), grids with attached substrates were placed on the bottom of the lake at a depth of 3 m. This depth was chosen both because it is the depth of maximum plankton productivity, indicating optimum light conditions for algal growth (J. Stanford, personal communication), and because it is beneath the heavy wave zone. Shade cloth was cleaned every 7–10 d by brushing off accumulated silt. Silt accumulation was minimal. After 27 d of exposure, each substrate was collected individually by a SCUBA diver, who removed the substrate from the grid, sealed it in a Ziplock® bag, and brought it to the surface. Once all were collected, substrates were immediately transferred to the laboratory. Periphyton was scraped from the top of the dish with a toothbrush. Samples were brought to constant volume and preserved in a 3% formaldehyde solution. The presence of grazers and grazer tracks was also recorded. Observed grazers included small snails, caddisflies, and midges. Evidence of grazers was apparent on less than 10% of the substrates and was not correlated with any treatment.

Light levels were determined in situ on August 10, 1988, at 2:00 p.m. using a Licor® submarine photometer. Percent transmission of incident photosynthetically active radiation (PAR) was measured with a deck cell and a spherical underwater sea cell. A SCUBA diver held a piece of each type of shade cloth over the sea cell to determine PAR values for the shade treatments. Light levels were 1840 $\mu einsteins \cdot cm^{-2} \cdot s^{-1}$ at the water surface, 720 $\mu einsteins \cdot cm^{-2} \cdot s^{-1}$ on the unshaded treatments, 430 $\mu einsteins \cdot cm^{-2} \cdot s^{-1}$ on medium-shade substrates, and 270 $\mu einsteins \cdot cm^{-2} \cdot s^{-1}$ on high-shade substrates. These readings indicated that the medium- and high-shade treatments reduced light intensity by approximately 40 and 63%, respectively. Because these in situ measurements were highly variable (due to motion of the boat), further light data were generated in the laboratory using a Hewlett Packard 8452A diode array spectrophotometer. A piece of shade cloth was placed in front of the light source and percent transmittance spectra for wavelengths between 300 and 800 nm were generated. Each screen was randomly moved 10 times and 10 spectra were generated for each shade treatment. Mean values of percent transmittance of selected wavelengths were calculated for each treatment. The wavelengths selected corresponded with peak absorbance values of common algal pigments. Light readings generated in the laboratory showed larger differences among treatments. The mean transparency value for the medium-shade treatment was 58% (SE = 0.32) of incident light which was consistent with values generated in the field for this treatment. However, the mean transparency value for the high-shade treatment was 27% (SE = 4.1), lower than that generated in the lake. The mean values of transparency that were generated in the laboratory differed significantly between shade treatments (Kruskal–Wallis χ^2 approximation = 147.1, df = 1, $p < 0.0001$, $n = 220$). Percent transmittance across the visible light spectrum was not different for either shade treatment (Kruskal–Wallis, $p = 0.994$). Thus, only light intensity, and not light quality, differed among treatments.

Nutrient release rates from nutrient-diffusing substrates reported in other studies (Fairchild et al. 1985; Bauman 1988) demonstrate that significantly different nutrient regimes are attained with this method. Most other factors (temperature, pH, etc.) were experimentally controlled, with the exception of gra-

TABLE 1. Three-way ANOVA and a posteriori comparisons assessing the variation of periphyton in three nitrogen, three phosphorus, and three shade treatments. Biovolumes and cell densities on treatments are ranked in increasing order from left to right. Treatments joined by underlining did not differ significantly ($P < 0.03$).

Dependent variable	Source	*F*-value	*p*-value	Tukey test
Total biovolume	Nitrogen	37.48	0.0001	Low Med, High
	Phosphorus	5.80	0.0049	Low High, Med.
	Shade	1.24	0.2973	Unshaded Med, High
	Nitrogen × phosphorus	5.88	0.0004	
	Nitrogen × shade	1.47	0.2222	
	Phosphorus × shade	2.32	0.0664	
Algal cell density	Nitrogen	47.57	0.0001	Low Med, High
	Phosphorus	4.14	0.0206	Low Med, High
	Shade	5.50	0.0063	Med. High Unshaded
	Nitrogen × phosphorus	2.74	0.0362	
	Nitrogen × shade	2.37	0.0622	
	Phosphorus × shade	1.79	0.1429	
Diatom biovolume	Nitrogen	37.47	0.0001	Low Med, High
	Phosphorus	6.27	0.0033	Low High, Med.
	Shade	0.96	0.3904	Med. High Unshaded
	Nitrogen × phosphorus	6.20	0.0003	
	Nitrogen × shade	1.53	0.2054	
	Phosphorus × shade	2.36	0.0629	
Diatom cell density	Nitrogen	58.14	0.0001	Low Med, High
	Phosphorus	1.99	0.1449	Low Med. High
	Shade	4.41	0.0162	Med. High Unshaded
	Nitrogen × phosphorus	4.35	0.0036	
	Nitrogen × shade	2.08	0.0940	
	Phosphorus × shade	1.82	0.1368	
Green algal biovolume	Nitrogen	14.55	0.0001	Low Med, High
	Phosphorus	0.32	0.7290	Low High Med.
	Shade	3.23	0.0464	Med. High Unshaded
	Nitrogen × phosphorus	2.20	0.0790	
	Nitrogen × shade	1.08	0.3721	
	Phosphorus × shade	0.81	0.5217	
Green algal cell density	Nitrogen	23.04	0.0001	Low Med, High
	Phosphorus	1.09	0.3436	Low High Med.
	Shade	3.94	0.0245	Med. High Unshaded
	Nitrogen × phosphorus	3.56	0.0112	
	Nitrogen × shade	0.62	0.6470	
	Phosphorus × shade	1.58	0.1901	
Blue-green algal biovolume	Nitrogen	0.20	0.8219	High Med, Low
	Phosphorus	0.66	0.5232	Low High Med.
	Shade	4.66	0.0136	High Med. Unshaded
	Nitrogen × phosphorus	0.32	0.8603	
	Nitrogen × shade	1.54	0.2037	
	Phosphorus × shade	1.25	0.3018	
Blue-green algal cell density	Nitrogen	0.29	0.747	Med. High Low
	Phosphorus	1.11	0.337	Low High Med.
	Shade	3.96	0.024	High Med. Unshaded
	Nitrogen × phosphorus	0.43	0.785	
	Nitrogen × shade	1.73	0.155	
	Phosphorus × shade	0.91	0.466	

zer densities. Few grazers were observed on or near the substrates.

Periphyton was enumerated in a Palmer–Maloney nanoplankton counting chamber at 450× magnification. The number of cells counted ranged from 500 to 1500 per sample. All algae were identified to genus or species in the counting chamber with the exception of pennate diatoms, which were determined from standard Hyrax® mounts (Patrick 1966) using a Leitz Dialux research microscope at 1000× under oil immersion. Cell densities were converted to biovolume by determin-

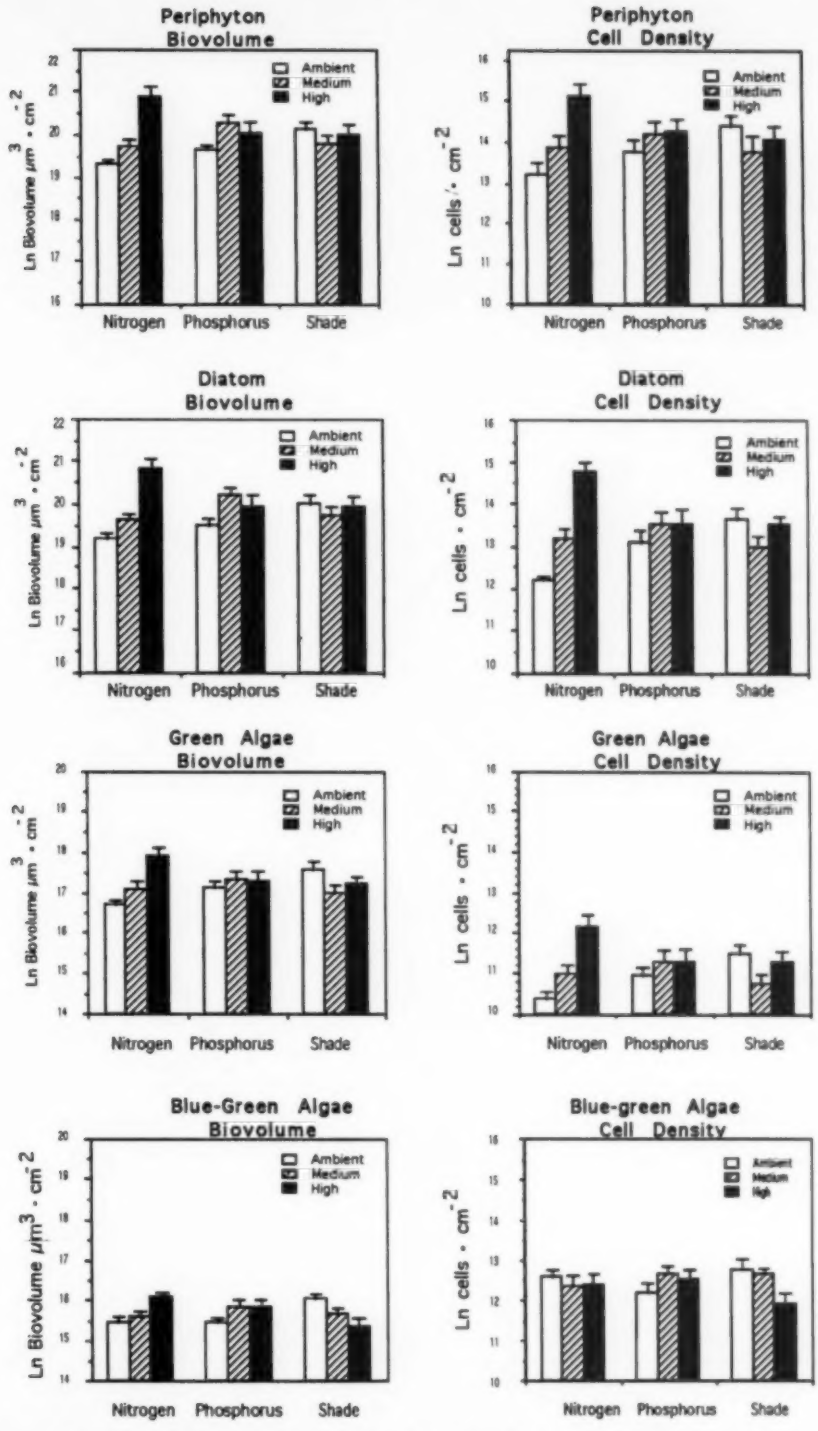

FIG. 1. Mean biovolumes and cell densities of total periphyton, diatoms, green algae, and blue-green algae as functions of nitrogen, phosphorus, and shade treatments. For nitrogen treatments, ambient = 0.0 M, medium = 0.05 M, high = 0.50 M; for phosphorus treatments, ambient = 0.0 M, medium = 0.05 M, high = 0.50 M; for light treatments, ambient = no shade cloth, medium = light shade cloth, high = heavy shade cloth. Vertical bars denote 1 SE of the mean; $n = 27$ for each treatment.

ing cell dimensions of 10 randomly chosen cells of each species and incorporating these dimensions into formulae for volumes of appropriate geometric shapes. Relative abundances of taxa were calculated based on biovolumes. We attempted to analyze microinvertebrate densities across treatments; however, they were not abundant enough to perform statistical tests.

Statistical analyses were performed on cell densities and biovolumes. Data were analyzed using a three-way analysis of variance (ANOVA) with nitrogen, phosphorus, and light as main effects. Data were transformed when necessary to meet the assumptions of the test. Tukey's multiple range test was used to test for pairwise differences among treatments. All analyses were performed using Statistical Analysis Systems (SAS Institute 1982). Due to the problems that arise from multiple statistical tests on the same data set (ambiguous alpha levels), a predetermined alpha value of 0.03 was used in assessing statistically significant differences from ANOVA tests. This reduced the chance of a type I error (Zar 1984). All significant differences reported herein are statistically significant at a probability level of <0.03 unless otherwise noted.

Results

Estimates of Algal Biomass

Algal biovolume increased with both nitrogen and phosphorus enrichment (Table 1; Fig. 1). Nitrogen had a stronger effect on enhancing periphyton growth than did phosphorus (Fig. 1). There was also a significant interactive effect between nitrogen and phosphorus (Table 1; Fig. 2). At low nitrogen levels (0.0 M), phosphorus enrichment had no effect; at medium nitrogen levels (0.05 M), periphyton biovolume was highest on the medium-phosphorus substrates (0.05 M); at high nitrogen levels (0.5 M), biovolume accumulation was highest on the high-phosphorus substrates (0.05 M). Periphyton biovolume did not differ significantly among light treatments (Table 1; Fig. 1). Differences in cell numbers among nutrient treatments showed trends similar to biovolume with respect to nutrient enrichment (Table 1; Fig. 1). However, unlike biovolume, cell numbers responded negatively to shading (Table 1; Fig. 1).

Division-level Responses

Diatoms were the dominant algal division by biovolume across all treatments (mean relative abundance 89.8 ± 6.3%) followed by green algae (mean relative abundance 8.1 ± 5.6%) and blue-green algae (mean relative abundance 2.1 ± 1.8%). Both diatom biovolumes and cell densities increased with nitrogen enrichment (Table 1; Fig. 1). Diatom biovolume also increased with phosphorus enrichment, although the highest mean accrual was observed on the medium-phosphorus treatment (Table 1; Fig. 1). Diatom cell density did not differ significantly among phosphorus treatments (Table 1; Fig. 1). The interactive effect between nitrogen and phosphorus was present in both diatom biovolumes and cell densities (Fig. 2). Diatom cell densities differed across shade treatments (Table 1; Fig. 1); cell densities were highest on the unshaded substrates, followed by the highly shaded substrates and the medium-shaded substrates. Diatom biovolumes were similar across all light treatments.

Green algal biovolumes and cell densities increased with nitrogen enrichment (Table 1; Fig. 1 and 2). Green algal cell densities responded to light in the same manner as diatoms (unshaded > high shade > medium shade) (Table 1; Fig. 1). Biovolumes showed the same trend ($p = 0.046$) but were not statistically significant at the predetermined alpha level (0.03). Blue-green algae did not exhibit any changes in biovolume or cell density across either nitrogen or phosphorus treatments (Table 1; Fig. 1). Blue-green biovolumes and cell densities decreased with shading (unshaded > medium shade > high shade (Fig. 1)). Blue-greens were the only division to show a consistent negative effect of shading.

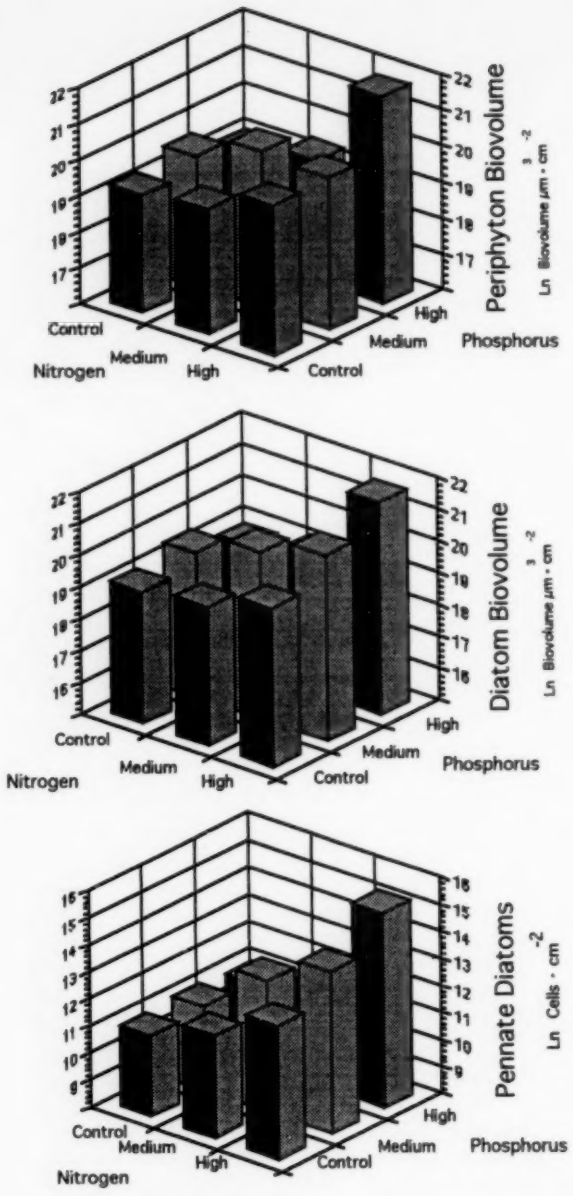

FIG. 2. Mean values of periphyton and diatom biovolumes and pennate diatom cell densities as functions of all combinations of three nitrogen and three phosphorus treatments. Treatment levels are as described in Fig. 1; $n = 9$ for each treatment.

TABLE 2. Algal taxa that responded to nutrient enrichment. Cell densities of taxa on the nutrient treatments are ranked in increasing order from left to right. Treatments joined by underlining did not differ significantly ($p < 0.03$).

Taxon	Source	p-value	Tukey test
	Algal taxa that responded only to nitrogen enrichment		
Cyclotella	Nitrogen	0.0015	Low Med. High
Cymbella	Nitrogen	0.0001	Low Med. High
Epithemia spp.	Nitrogen	0.0286	High Med. Low
Melosira varians	Nitrogen	0.0004	Low Med. High
Schizothrix	Nitrogen	0.0001	Low Med. High
Tabellaria	Nitrogen	0.0127	Med. Low High
Ulothrix	Nitrogen	0.0005	Low Med. High
	Algal taxa that responded to nitrogen and showed a significant interaction between nitrogen and phosphorus		
Achnanthes minutissima	Nitrogen	0.0001	Low Med. High
	Phosphorus	0.6093	Low Med. High
	Nitrogen × phosphorus	0.0219	
Cymbella microcephala	Nitrogen	0.0017	Low Med. High
	Phosphorus	0.4408	High Med. Low
	Nitrogen × phosphorus	0.0387	
Geminella sp.	Nitrogen	0.0001	Low Med. High
	Phosphorus	0.9656	Low High Med.
	Nitrogen × phosphorus	0.0008	
	Algal taxa that responded to both nitrogen and phosphorus		
Pennate diatoms	Nitrogen	0.0001	Low Med. High
	Phosphorus	0.0085	Low High Med.
	Nitrogen × phosphorus	0.0037	
Rhopalodia gibba	Nitrogen	0.0041	High Med. Low
	Phosphorus	0.0073	Low High Med.
	Nitrogen × phosphorus	0.5311	
Scenedesmus sp.	Nitrogen	0.0001	Low Med. High
	Phosphorus	0.0311	Low High Med.
	Nitrogen × phosphorus	0.0228	

Taxon-level Responses

Because of the high diversity of algal species in Flathead Lake, only the responses of abundant species (relative abundance >1%) that were found on at least 85% of the substrates were analyzed. Taxa analyzed were identifiable from the Palmer cell analysis. Most analyses are at the generic level, although some species-level responses will be presented. In addition, pennate diatoms that were not identifiable from the Palmer cell analyses have been grouped together and treated as a taxon. Examination of the cleaned diatom slides revealed that these diatoms were mostly (>90%) five species: *Nitzschia palea, Nitzschia dissipata, Synedra rumpens, Synedra ulna*, and *Fragilaria vaucheriae*. These diatoms responded to treatments in a similar manner. *Nitzschia palea* was the dominant species in this group.

Six taxa were enhanced only by nitrogen enrichment (Table 2). Three taxa were stimulated by nitrogen and showed an interactive effect between nitrogen and phosphorus, although they did not respond independently to phosphorus (Table 2). Two taxa, *Scenedesmus quadricauda*, a colonial green alga, and pennate diatoms, were enhanced by both nitrogen and phosphorus and showed an interactive effect between nitrogen and phosphorus (Table 2). Cell densities of *Scenedesmus* and pennate diatoms were higher on the medium-phosphorus substrates than on the high-phosphorus and ambient-phosphorus substrates. Neither population responded to phosphorus as strongly as to nitrogen. The interactive effect of nitrogen and phosphorus again illustrated that phosphorus enrichment had a stronger effect when nitrogen levels were increased (Fig. 2).

Both *Rhopalodia gibba* and *Epithemia*, two large diatoms of the family Epithemiaceae, experienced decreases in cell density with nitrogen enrichment (Table 2; Fig. 3). Due to the large size of these taxa, small changes in cell densities resulted in dramatic changes in species composition as measured by biovolume. *Rhopalodia gibba* responded significantly to phosphorus enrichment. The highest mean cell density of *R. gibba* was on the medium-phosphorus substrates. Cell densities on the high-phosphorus substrates could not be differentiated from those on the unenriched substrates.

Five taxa had significantly higher cell densities and relative abundances on unshaded substrates (Table 3). Cell densities of *Cyclotella* and *Anabaena* were lowest on the high-shade substrates whereas cell densities of *Geminella, Melosira varians*, and pennate diatoms were lowest on the medium-shade treat-

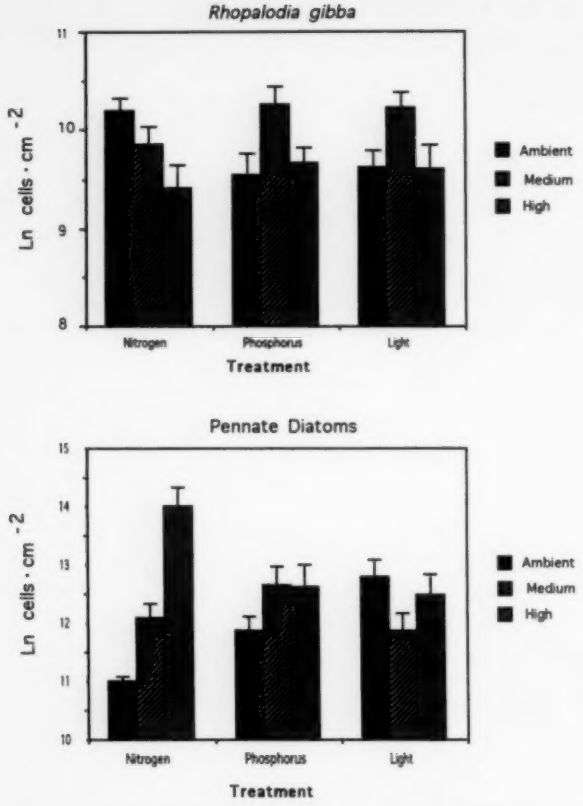

FIG. 3. Mean cell densities of *R. gibba* and pennate diatoms as functions of nitrogen, phosphorus, and shade treatments. Treatment levels are as described in Fig. 1. Vertical bars denote 1 SE of the mean; $n = 27$ for each treatment.

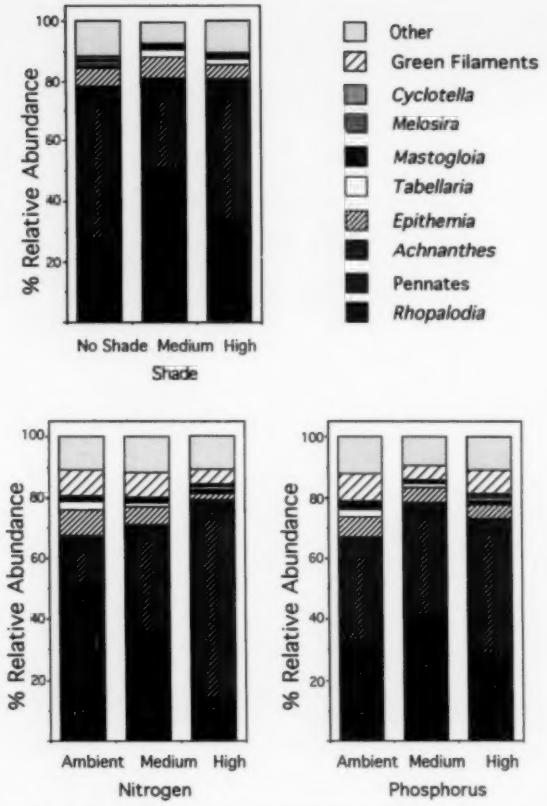

FIG. 4. The relative contribution to total biovolume of selected algal taxa across three nitrogen, three phosphorus, and three light treatments. Treatment levels are as described in Fig. 1.

TABLE 3. Algal taxa that showed significant responses to shade treatments. Cell densities of taxa on the three shade treatments are ranked in increasing order from left to right. Treatments joined by underlining did not differ significantly ($p < 0.03$).

Taxon	Source	*p*-value	Tukey test
Anabaena	Shade	0.0002	High Med. Unshaded
Cyclotella	Shade	0.0052	High Med. Unshaded
Geminella	Shade	0.0008	Med. High Unshaded
Melosira varians	Shade	0.0216	Med. High Unshaded
Pennate diatoms	Shade	0.0037	Med. High Unshaded
Rhopalodia	Shade	0.0132	High Unshaded Med.

ments. In contrast, cell densities and relative abundances of *R. gibba* were highest on the medium-shade substrates (Table 3).

Despite the high diversity of algal taxa in Flathead Lake, most of the changes in assemblage structure across treatment levels were precipitated by strong changes in the abundance of *R. gibba* and pennate diatoms (Fig. 3 and 4). The mean relative biovolume of pennate diatoms increased from 14.3% on low-nitrogen substrates to 58.5% on high-nitrogen substrates. In contrast, the mean relative biovolume of *R. gibba* decreased from 60.4% on nitrogen controls to 16.5% on high-nitrogen substrates. Changes in species composition due to phosphorus enrichment were not as striking as those due to nitrogen and were confined to three algal taxa. The proportion of pennate diatoms increased with phosphorus levels, and the proportions of green filaments and *Achnanthes* decreased on the medium-phosphorus substrates. The relative abundances of *Rhopalodia* and *Epithemia* did not vary significantly with phosphorus treatments. Differences across light treatments were also caused by shifts in dominance between pennate diatoms and *R. gibba* (Fig. 4).

Discussion

Nitrogen enrichment provoked a much stronger response than phosphorus enrichment, suggesting that nitrogen is more limiting than phosphorus to this periphyton assemblage. As predicted, the interactive effect between nitrogen and phosphorus indicated that nitrogen was the primary limiting nutrient, but when nitrogen became abundant, phosphorus became limiting. In contrast, Bauman (1988) performed a similar experiment in Flathead Lake and found that phosphorus enrichment had a stronger effect than nitrogen in stimulating periphyton. Nutrient fluxes may vary annually in Flathead Lake as may the effects of anthropogenic disturbance. Because ambient concentrations of both nitrogen and phosphorus are so low in Flathead Lake, point-source nutrient loading could have large local impacts on

nutrient regimes, including nitrogen to phosphorus ratios for periphyton.

Studies on phytoplankton in Flathead Lake have reported that the phytoplankton were primarily phosphorus limited, although there was also an additive effect of nitrogen enrichment (Stanford et al. 1983; C. Spencer, unpubl. data). Because much of the phosphorus introduced into Flathead Lake becomes bound in the sediments, it may be more available to periphyton than to phytoplankton, which may account for the discrepancy in nutrient limitation between the periphyton and the phytoplankton.

The increase in biovolume due to nitrogen enrichment was primarily caused by increases in pennate diatoms and *Achnanthes minutissima*. The presence of many of these taxa in nutrient-rich habitats is well documented (Palmer 1969; Lowe 1974; Stockner and Shortreed 1978; Fairchild et al. 1985; Carrick and Lowe 1988). Possible mechanisms for the negative effect of nitrogen enrichment on *R. gibba* are that it was either physically displaced by pennate diatoms that were actively dividing on nutrient-enriched substrates or that pennate diatoms drove local phosphorus levels below the minimal requirement for *R. gibba*. Shading of *R. gibba* by pennate diatoms is an unlikely mechanism, given this taxon's response to light (Fig. 3).

The increases in biovolume and cell density with phosphorus enrichment can be attributed primarily to increases in *R. gibba* on ambient-nitrogen substrates and increases in pennate diatoms on nitrogen-enriched substrates. *Rhopalodia gibba* was the only taxon to respond positively to the addition of phosphorus alone. This diatom possesses endosymbiotic blue-green algae that are capable of fixing nitrogen (Floener and Bothe 1980). *Rhopalodia gibba* and other species in the family Epithemaceae have previously shown enhanced growth on high-phosphorus substrates, which has been attributed to the ability of these species to fix nitrogen in a nitrogen-poor habitat (Fairchild and Lowe 1984; Fairchild et al. 1985). Lake and laboratory studies show that the number of endosymbionts per diatom varies with external nutrient concentrations. Endosymbiont load decreases as nitrogen increases, but increases with phosphorus enrichment (DeYoe et al. 1992). This mechanism probably permits these diatoms to thrive in low-nitrogen, high-phosphorus habitats.

Elevated algal biovolume on medium-phosphorus substrates relative to high-phosphorus substrates has been previously observed (Fairchild and Lowe 1984; Hill and Knight 1988) and suggests an inhibitory effect of the high-phosphorus treatments on some taxa. Inhibition due both to excess phosphorus (Rodhe 1948) and to high concentrations of potassium (Lehman 1976) has been previously documented. The high-phosphorus treatments had a negative effect on biovolume on the low-nitrogen and the medium-nitrogen substrates whereas biovolume increased on the high-phosphate substrates when nitrogen levels were high. Thus, when nitrogen is limiting, phosphorus treatments or the associated potassium appear to be inhibitory.

The lack of response of periphyton biovolume to shade treatments was counter to the initial hypothesis that periphyton accrual would be decreased by shading. These findings contradict other studies that demonstrated that periphyton accrual was correlated with light availability in lentic systems (Stevenson and Stoermer 1981; Hudon and Bourget 1983; Hoagland and Peterson 1990). Studies performed in shaded or artificial streams that showed decreased algal biomass with shading (Lowe et al. 1986; Steinman and McIntire 1986; Hill and Knight 1988; Steinman et al. 1989) included light levels much lower (50 μeinsteins$\cdot m^{-2} \cdot s^{-1}$) than the highest shade treatment in this experiment. Thus, despite a 70% reduction in light intensity, light levels were well within the range in which algal photosynthesis appears to be light saturated. Shade cloths may have inhibited algal immigration from the water column; however, this would tend to bias the results towards detecting a negative effect due to shading.

Based on prior studies, we had anticipated that green filaments, which tend to require higher light levels than diatoms, would thrive on the high-nutrient, high-light substrates (Hansmann and Phinney 1973; Shortreed and Stockner 1983; Lowe et al. 1986; Marks and Lowe 1989). It is unclear why green filaments did not thrive under these conditions. There may not be many suitable microhabitats for green filaments in Flathead Lake and consequently a deficient "spore bank" of green filaments to colonize the substrates. Two green filaments, *Stigeoclonium* and *Ulothrix*, were present on high-nutrient substrates, but neither taxon established large populations. Perhaps if the experiment had been continued for a longer time period, green filaments would have come to dominate high-resource substrates. This in turn might have resulted in a significant light effect. Alternatively, green filaments could have been limited by another factor not manipulated in this experiment.

Blue-green algae were the only major taxonomic group to respond to light manipulations. Because the relative abundance of blue-greens never exceeded 7.9%, the reduction in their abundance on shaded substrates did not result in profound changes in assemblage structure. It is interesting to note that the blue-green filaments *Tolypothrix* and *Scytonema*, which covered rocks in shallower areas of the lake, were not found on rocks at 3 m. These filaments could be light limited at these depths. This may be why the diatoms *Rhopalodia* and *Epithemia*, rather than blue-greens, dominate rocks below a few metres.

Achnanthes minutissima was the only taxon that had a significantly higher relative abundance in the high-shade treatment. This small taxon has also been reported to thrive in areas of limited space and light (Pringle and Bowers 1984). On nitrogen-enriched substrates, it was observed growing in clumps, on stalks, and as an epiphyte on other algal filaments. This physiognomy could be a mechanism for finding suitable light and space within a crowded periphyton matrix (Hoagland et al. 1982). Although the interaction term between nitrogen and light was not statistically significant ($p = 0.049$), it appears that when nutrient concentrations are high, *A. minutissima* can thrive when light levels are low. Further work on the physiognomy of this taxon under different nutrient and light regimes would be interesting because it has the ability to grow in both an adnate and a stalked form. The adnate form could be advantageous for acquiring nutrients from the sediments whereas the stalked form could be advantageous for acquiring light once a thick periphyton mat has been established.

The discrepancies between biovolume and cell density reflect a perpetual problem in analyzing periphyton communities. Large increases in small taxa such as *A. minutissima* are often not revealed in a biovolume analysis. However, their contribution to primary production could be very important and hence underestimated by this type of analysis. Small taxa that are dividing quickly could be more important to higher trophic levels than larger taxa, which despite their contribution to biovolume are not as physiologically active. Investigations of interactions between trophic levels in aquatic systems support

this idea and have shown that actively dividing cells have lower carbon to nitrogen ratios (McMahon et al. 1974; Hunter 1980). Actively dividing cells have a relatively high protein content and are preferred by grazers.

We have shown that resolution of the responses of different algal genera is useful in interpreting the independent and interactive responses of periphyton to nutrient and light resources. Although most of the differences between treatments were the result of changes in the populations of a few major taxa, understanding mechanisms of population growth for these taxa is integral to predicting changes in algal biomass. For example, differences among diatoms that do or do not possess nitrogen-fixing endosymbionts, or differences in species' abilities to rapidly reproduce following nutrient pulses, can account for differential responses of these taxa to nitrogen and phosphorus. Further study of the distinct physiological and morphological characteristics of algae will repay investigators with a more detailed understanding of their ecological responses to resource changes in their environment.

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Direct and Indirect Effects of Fish Predation on the Replacement of a Native Crayfish by an Invading Congener

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In Ohio streams, the crayfish *Orconectes rusticus* is replacing *O. sanborni*, and herein we test how predators influence this replacement. In a field survey, crayfish were scarce when fish were abundant, suggesting that predators can adversely affect these prey. In laboratory experiments, we examined underlying mechanisms for this inverse relationship; specifically, we tested how crayfish species, adult aggression, and habitat heterogeneity influenced the predator–prey interaction. In a laboratory stream, smallmouth bass (*Micropterus dolomieu*) ate similar numbers of equal-sized *O. rusticus* and *O. sanborni*, but when sizes mimicked those in the field (i.e., *O. rusticus* 4 mm > *O. sanborni*), fewer *O. rusticus* were eaten. Fish also reduced juvenile activity and behaviors whereas adult aggression increased the frequency of these risky responses. More affected by adult crayfish, *O. sanborni* should suffer disproportional predation where adults and juveniles interact. Thus, fish predators should increase replacement rates and adult aggression should further accelerate this process. Manifested through crayfish size, both indirect and direct predator effects contribute to the replacement of *O. sanborni* by *O. rusticus*.

Dans les cours d'eau de l'Ohio, l'écrevisse *Orconectes rusticus* remplace *O. sanborni*, et nous avons examiné la façon dont la prédation influe sur ce phénomène. Dans une étude menée sur le terrain, les écrevisses étaient rares quand les poissons étaient abondants, ce qui semble indiquer que les prédateurs peuvent avoir un effet négatif sur la présence des crustacés. Dans les expériences de laboratoire, nous avons examiné les mécanismes qui sous-tendent cette relation inverse; plus précisément, nous avons étudié la façon dont les espèces d'écrevisses, l'agression par les adultes et l'hétérogénéité de l'habitat influent sur l'interaction entre prédateurs et proies. Dans un cours d'eau artificiellement recréé en laboratoire, les achigans à petite bouche (*Micropterus dolomieu*) mangeaient des nombres similaires de *O. rusticus* et de *O. sanborni* de taille égale, mais quand on reproduisait les tailles existant dans la nature (c.-à-d. *O. rusticus* mesurant 4 mm > *O. sanborni*), le nombre de *O. rusticus* consommées était inférieur. Les poissons réduisaient ainsi l'activité des juvéniles et leurs comportements, tandis que l'agression par les adultes augmentait la fréquence de ces réactions à risque. Davantage affectée par l'action des écrevisses adultes, *O. sanborni* doit subir une prédation disproportionnée quand les adultes et les juvéniles sont en interaction. Ainsi, la prédation par les poissons doit accentuer le remplacement d'une espèce par l'autre, et l'agression par les adultes doit accélérer ce processus. Par le biais de la taille des écrevisses, les effets indirects et directs de la prédation contribuent au remplacement de *O. sanborni* par *O. rusticus*.

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In central Ohio streams, a crayfish species replacement is occurring and provides a unique opportunity to explore processes that underlie species interactions and extirpations. *Orconectes rusticus* is a large, aggressive crayfish (Capelli 1982; Butler and Stein 1985) that naturally occurs in Indiana, Kentucky, and western Ohio. This invader is becoming increasingly common outside its native range, probably as a result of baitbucket introductions (Capelli 1982), and has been associated with declines of native crayfish in Ontario (Berrill 1978) and Wisconsin (Capelli 1982). Within Ohio, *O. rusticus* has continued to expand its eastern boundary (Turner 1926; Rhoades 1962) and has displaced the native crayfish *O. sanborni* in parts of the Licking River (Butler and Stein 1985). In the replacement of native crayfishes by *O. rusticus*, the roles of abiotic factors (Rhoades 1944, 1962; Capelli and Magnuson 1983; Flynn and Hobbs 1984), reproductive interference (Tierney and Dunham 1984; Butler and Stein 1985), competition (Capelli and Munjal 1982; Butler and Stein 1985; Lodge et al. 1986), and community interactions (Olsen et al. 1991) have been examined. These crayfish replacements, although frequently documented, are difficult to study, inadequately understood, and appear to be a complex interaction among multiple factors (Lodge et al. 1986; Olsen et al. 1991).

Predation affects community structure in many aquatic systems (Sih et al. 1985), and juvenile crayfish are especially vulnerable to fish predators (Stein and Magnuson 1976; Stein 1977; Rabeni 1992). Elsewhere, we have described how differences in habitat-specific growth and overall mortality contribute to this replacement (Mather and Stein 1993). Here we test if fish predators play a role in the Ohio crayfish species replacement. For example, fish predators may affect crayfish either directly by consuming them or indirectly by causing them to change behavior, alter activity patterns, or shift habitats. If either cray-

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fish species is more vulnerable to direct or indirect predator effects and if these effects result in reductions in survival, fecundity, or growth, then fish predation can contribute to the replacement.

Herein we combine a field survey with laboratory experiments to ask the following specific questions: (1) Do stream fish predators limit the abundance of crayfish? (2) Does a common fish predator, smallmouth bass (*Micropterus dolomieu*), differentially influence vulnerability, activity, behavior, or habitat use of the two crayfishes in a simulated laboratory stream? (3) Does aggression of adult crayfish or habitat heterogeneity modify these responses? (4) Do any of these factors differentially affect either species when crayfish size reflects natural size distributions? (5) Do fish predators contribute to this ongoing replacement?

Methods

Field Survey

To assess the general relationship betwen predator and prey, we sampled fish and crayfish in five allopatric *O. sanborni* sites and six sympatric *O. sanborni*/*O. rusticus* sites ($N = 11$). These sites, 6–24 m wide ($\bar{X} = 12$ m), 0.5–0.9 m average depth ($\bar{X} = 0.6$ m), 0.7–1.5 m maximum depth ($\bar{X} = 1.0$ m), were located in four second-order streams in two drainages. Limited availability of sympatric sites prevented us, in this field study, from assessing the response of each crayfish species to predation, but supplementing sympatric with allopatric sites, at least, allowed us to examine the effect of fish on both crayfish species combined.

To determine crayfish density, we randomly chose three to five locations in each riffle and adjacent pool for quantitative kick seines (total = 6–10 samples in each riffle/pool combination). When crayfish movements were monitored over 24 h, only one crayfish attempted to change habitat (True 1990); thus, for our sampling, we assumed that crayfish remained in either riffle or pool. To sample, one person held the 1.5-m-wide seine while two others quickly and vigorously kicked 1 m upstream of the seine. All rocks were overturned swiftly to dislodge crayfish and the seine was quickly pulled through the 1.5-m^2 sample area. Crayfish were identified to species, sexed, and measured (nearest millimetre carapace length (CL)). All sites were sampled for crayfish within 10 d in late June 1987. Several survey sites were in the same tributary, but all pool samples were separated by at least 100 m or a riffle, obstacles adequate to ensure that crayfish populations at each site did not overlap.

To determine fish density, we sampled these same pools during 25 June through 5 July 1987. Blocknets were placed between (1) riffle and pool and (2) 35 m into the pool (about 560 m^2 of pool per site) ensuring that a representative area of both large and small pools was sampled. The area within the two blocknets was electroshocked twice ($\bar{X} = 22.5$ min/pass) with a pulsed DC unit. Upon capture, fish were identified to species, measured, and stomachs of all fish >100 mm total length (TL) were pumped (Seaburg 1957). Because only smallmouth bass and rock bass (*Ambloplites rupestris*) ≥150 mm contained crayfish, we limited our analysis to these larger predators. Riffles were not sampled because fish predators were never observed in these shallow-water habitats. For the same reason, only crayfish densities in pools were used in the analysis that follows.

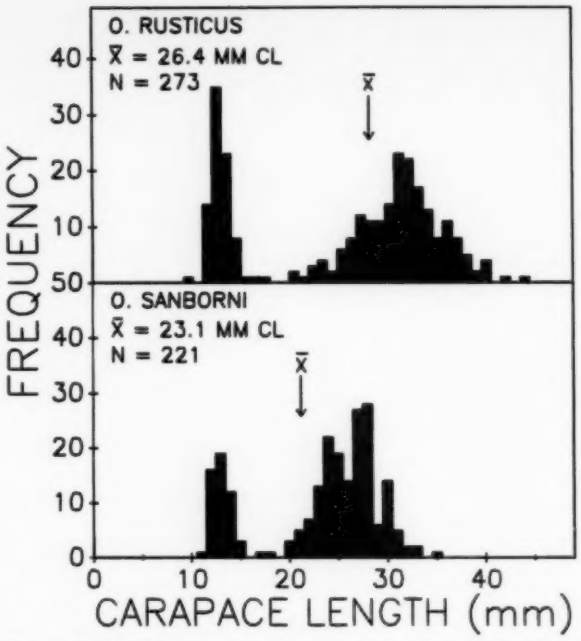

FIG. 1. Size distributions of *O. rusticus* and *O. sanborni* in North Fork Creek, Licking and Knox counties, Ohio, during June through September 1988.

Laboratory Experiments

Once a general relationship between fish and crayfish abundances was established by field sampling, we examined potential mechanisms for this replacement by examining the relative response of each crayfish species to predators in the laboratory. Specifically, in a stream tank, we quantified how fish predators influenced juvenile crayfish survival and behavior and how crayfish species, adult aggression, and habitat heterogeneity altered this predator–prey interaction. Our stream tank had two identical, independent, 2.6 × 0.9 × 1.5 m channels. Each channel contained a paddlewheel adjusted to generate bottom currents of 30 cm/s upstream to 1 cm/s downstream and included (1) a 0.9 × 0.9 × 0.06 m (deep) upstream riffle and (2) a 0.9 × 0.9 × 0.6 m (deep) downstream pool connected by (3) a 0.9 × 0.8 m transitional area (depth range = 0.06–0.6 m). Substrate was 5-mm dark pebble glued to Plexiglas to preclude crayfish burrowing. To eliminate chemical effects, water (16°C) was changed between experiments. A 14 h light: 10 h dark photoperiod with simulated dawn and dusk allowed us to observe diurnal, nocturnal, and crepuscular interactions in each 21-h experiment.

Treatments

We used a complete randomized 4 × 2 block design with the following treatments: (1) fish ($N = 1$ or none), (2) species (*O. rusticus*, *O. sanborni*), (3) adults ($N = 6$ *O. rusticus* males (i.e., 26 crayfish per experiment) or no adults (i.e., 20 crayfish per experiment)), and (4) shelters ($N = 10$ pool shelters or none). Fish predation, known to influence crayfish distribution and abundance (Stein and Magnuson 1976; Stein 1977; Rabeni 1992), occurs continuously in the field; to detect predation within a 21-h experiment, we used a higher fish density

(0.33/m^2) in the laboratory than normally observed in the field (see Fig. 2). When testing species in the laboratory, we focused on sympatric interactions because relative performance of each species is critical to understanding the replacement.

Third, we tested the effect of adult crayfish on juveniles because crayfish size affects both inter- and intraspecific interactions (Butler and Stein 1985; Rabeni 1985), and juvenile crayfish probably encounter ubiquitous adult crayfish frequently ($\bar{X}$ = 3/m^2). In preliminary experiments, juvenile crayfish responded to adults of both species similarly (ANOVA, $p > 0.05$); consequently, only easily obtained *O. rusticus* adults were used. To test how large adult crayfish affect the smallmouth bass/juvenile crayfish interaction, six adult males (≥25 mm CL, mostly form II) were used. Our experiments with form II males probably reflected natural levels of aggression but underestimated effects of more aggressive form I males.

Finally we quantified how habitat heterogeneity, via shelters, affected crayfish susceptibility to smallmouth bass predation because habitat heterogeneity changes the interaction between predators and prey (Huffaker 1958; Stein 1977; Brusven and Rose 1981; Savino and Stein 1982; Feltmate et al. 1986). We placed 10 single-crayfish shelters (5 × 2.5 × 3 cm pieces of slate supported by a 5-cm screw and sufficiently large to house a single juvenile) in pools. Experimental order within a block was random except that to prevent contamination by chemical cues, smallmouth bass were either present or absent from both channels.

Size Treatments

In the field, size distributions of the two crayfishes overlapped, but *O. rusticus* was larger than the displaced native *O. sanborni* (by 3.3 mm CL; Fig. 1). To evaluate the role of size, two sets of experiments testing the variables described above (fish predators, crayfish species, adult crayfish, habitat heterogeneity) were completed. In the first set of experiments (N = 8 replicates per treatment), conducted during September through December 1988, we used equal-sized juvenile crayfish ($\bar{X}$ = 15 mm CL). Juvenile crayfish of this size were common, large enough to mark, easy to handle, and readily consumed by experimental fish (250 mm TL). In the second set of experiments (N = 3 replicates per treatment), completed during September through December 1989, we used unequal-sized crayfish. To separate the effect of size and species in these unequal-size experiments, we continued to use about 15 mm CL ($\bar{X}$ = 15.5) as the small size; to this we added 4 mm (similar to 3.3 mm, the size difference observed in the field) to generate the larger juvenile size, 19.5 mm. Juveniles of these sizes (*O. rusticus*: $\bar{X}$ = 19.5 mm CL, *O. sanborni*: $\bar{X}$ = 15.5 mm CL, 4-mm size difference) may occur in the field between the end of their first summer (fast growers) and the end of their second summer (slow growers). Although 19.5 mm is not common (Fig. 1), all individuals must grow through this stage. In three quarters of the first experiments and two thirds of the second experiments, naive untested crayfish were used. Only in the last blocks were crayfish reused.

Experimental Animals

Collected from allopatric populations, each crayfish species was held separately in 625-L tanks and fed lettuce, fish, and liver ad libitum. Before experiments, each crayfish was measured and marked (2-mm epoxy paint mark on the carapace) to indicate species. In each experiment, we used 20 juvenile crayfish, 10 each of *O. rusticus* and *O. sanborni* (1:1 sex ratio), resulting in a combined density of 7/m^2, a density within the natural range found in Ohio streams (see Fig. 2). For simplicity, all small crayfish (15–19 mm CL) were termed juvenile. Smallmouth bass were collected from the Olentangy River, Franklin County, Ohio. Fish were maintained in individual 200-L tanks ($\bar{X}$ = 18°C) and fed minnows and crayfish ad libitum. Fish were starved 72–96 h and acclimated to 16°C for 24 h before each experiment. When equal-sized crayfish were used, smallmouth bass were 250–275 mm TL; in the following year when unequal-sized crayfish were used, fish were 320–350 mm TL.

General Protocol

Before experiments, habitats (riffle, transitional area, pool) were separated by screens. One smallmouth bass was placed in the transitional area between riffle and pool the evening before experiments to acclimate. The next morning, 4 h before an experiment, crayfish were divided equally between riffle and pool. Experiments began when screens were raised and fish and crayfish could interact. The following morning, 21 h into experiments, we lowered the screens, recorded final crayfish distribution, drained the tank, and recovered all surviving crayfish. After 1 and 20 h, crayfish activity, habitat choice, and agonistic behaviors were recorded. In the first 16 experiments, fish stomachs were pumped to verify that the number of crayfish eaten was the difference between the number added and the number recovered. For all these experiments, we could account for all crayfish, and consequently, we did not pump stomachs in the remaining experiments.

Response Variables

We measured six responses: number eaten, proportion active, habitat changes, swimming escapes, fights, and final habitat choice. Number eaten and final habitat choice were measured only at experiment's end. Other responses were quantified for 5 min in each habitat during two observation periods, 1 and 20 h (total observation time/experiment = 5 min/habitat × 3 habitats × 2 observation periods = 30 min/experiment). Crayfish responses at 1, 2, 3, and 4 h were similar (ANOVA, $p > 0.05$); hence, the 1-h observation period was chosen to depict early crayfish interactions. Because responses were similar for 1 and 20 h, these data were combined.

Proportion active was estimated by scan sampling each habitat. If a crayfish did not move for 5 s, it was considered inactive. Habitat changes occurred when a crayfish moved between riffle, transitional area, or pool. Fights included both contact and noncontact aggressive interactions, i.e., when a crayfish was located within one body length of and oriented toward another individual. Swimming escapes occurred when crayfish jumped off the bottom and swam or drifted away in response to a threat from another crayfish. To determine habitat choice, riffle and pool crayfish were counted at the end of experiments. Here, riffles were defined as the area from which fish were excluded, i.e., the shallow, fast riffle and the upstream 10 cm of the transitional area between riffle and pool. Functionally defined as the habitat the fish frequented, the pool included the deepest quadrant and the 70 cm of transitional area to which fish had access. Because pool/riffle comparisons were not made within an experiment, the size of these habitats was not standardized.

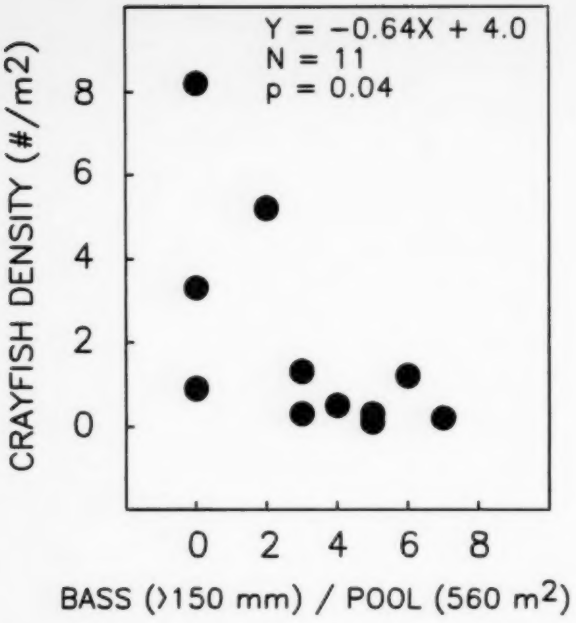

FIG. 2. Relationship between fish predator density (rock bass and smallmouth bass >150 mm TL·560 m^{-2} pool) and total crayfish density (all crayfish captured in three to five quantitative kick seines per site) in central Ohio streams in June and July 1987.

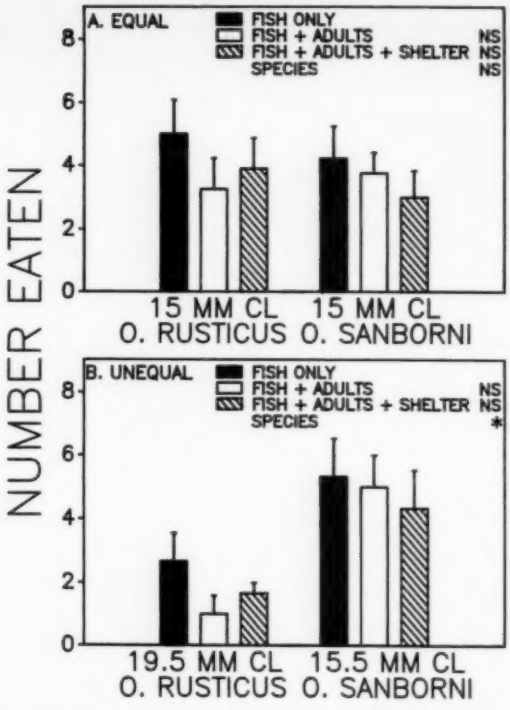

FIG. 3. Number of *O. rusticus* and *O. sanborni* eaten by smallmouth bass. (A) Both crayfish species were of equal size ($N = 8$); (B) crayfish were of unequal size, reflecting a natural size difference ($N = 3$). Data are means ±1 SE. A three-way ANOVA tested for effects of crayfish species, adult crayfish, and shelters on juvenile crayfish survival. NS, $p > 0.05$; *$p \leq 0.05$. Significant SS, F, and df are given in the text.

When fish ate crayfish, prey density differed at the beginning and the end of the experiment. Our experiments were not designed to separate behaviors of surviving crayfish from those that were eaten. Examining frequency of behaviors scaled to the initial number of crayfish incorporates all activities that occurred before crayfish were eaten; the alternative, reformulating each response as the proportion of the surviving population, accounts for the density change, but ignores initial behaviors. Because of the importance of prepredation activities, we chose to scale responses to the *initial* number of crayfish.

Statistical Analyses

Six four-way ANOVAs determined how species, fish, adults, and shelters influenced number of juveniles eaten, proportion active, habitat changes, swimming escapes, fights, and proportion in the riffle (SAS Institute, Inc. 1982). Number eaten was evaluated at the $p \leq 0.05$ level. The other five nonlethal responses were not independent, and to control experimentwise error, we evaluated each comparison at the $p \leq 0.05/5$ (0.05 for 5 responses) or $p \leq 0.01$ level (0.01 for each response). In preliminary experiments, juvenile crayfish responded similarly whether (1) all 20 juvenile crayfish were the same species or (2) species were mixed 1:1 *O. sanborni*/*O. rusticus* (ANOVA, $p > 0.05$). Because juvenile crayfish responded to density and not to species, species was considered a treatment effect. When an analysis of residuals revealed that a transformation was necessary to stabilize variances, proportions and behaviors were arcsin and log transformed, respectively. Only significant trends are discussed below unless explicitly noted otherwise.

Results

Direct, Lethal Fish Effects

In the field, fish predators did not occur in equal numbers at all sites. Across 11 sites, fish abundance varied from 0 to 7 fish/560 m^{-2} pool and crayfish density ranged from 0 to 8.3/m^{-2}. As predator abundance increased, total crayfish density declined (Fig. 2).

To determine if smallmouth bass negatively affect crayfish, as the field survey suggests, we quantified predator effects in the laboratory. In the stream tank, fish ate similar numbers of equal-sized *O. rusticus* and *O. sanborni* (Fig. 3A). When crayfish were of unequal-size (*O. rusticus* 4 mm > *O. sanborni*), fewer large *O. rusticus* were eaten than small *O. sanborni* (Fig. 3B: SS = 2.00, $F = 4.49$, df = 23, $p = 0.05$).

Indirect, Nonlethal Fish Effects

Without fish, the majority of *O. rusticus* and *O. sanborni* of both sizes were active (Fig. 4A, 4B). When unequal in size, *O. rusticus* was more active than *O. sanborni* (Fig. 4B: SS = 1.08, $F = 17.08$, df = 47, $p = 0.0002$). Fish reduced activity for both species and both sizes (Fig. 4A: SS = 1.66, $F = 41.06$, df = 127, $p = 0.0001$; Fig. 4B: SS = 2.42, $F = 8.33$, df = 47, $p = 0.0001$). When crayfish were of

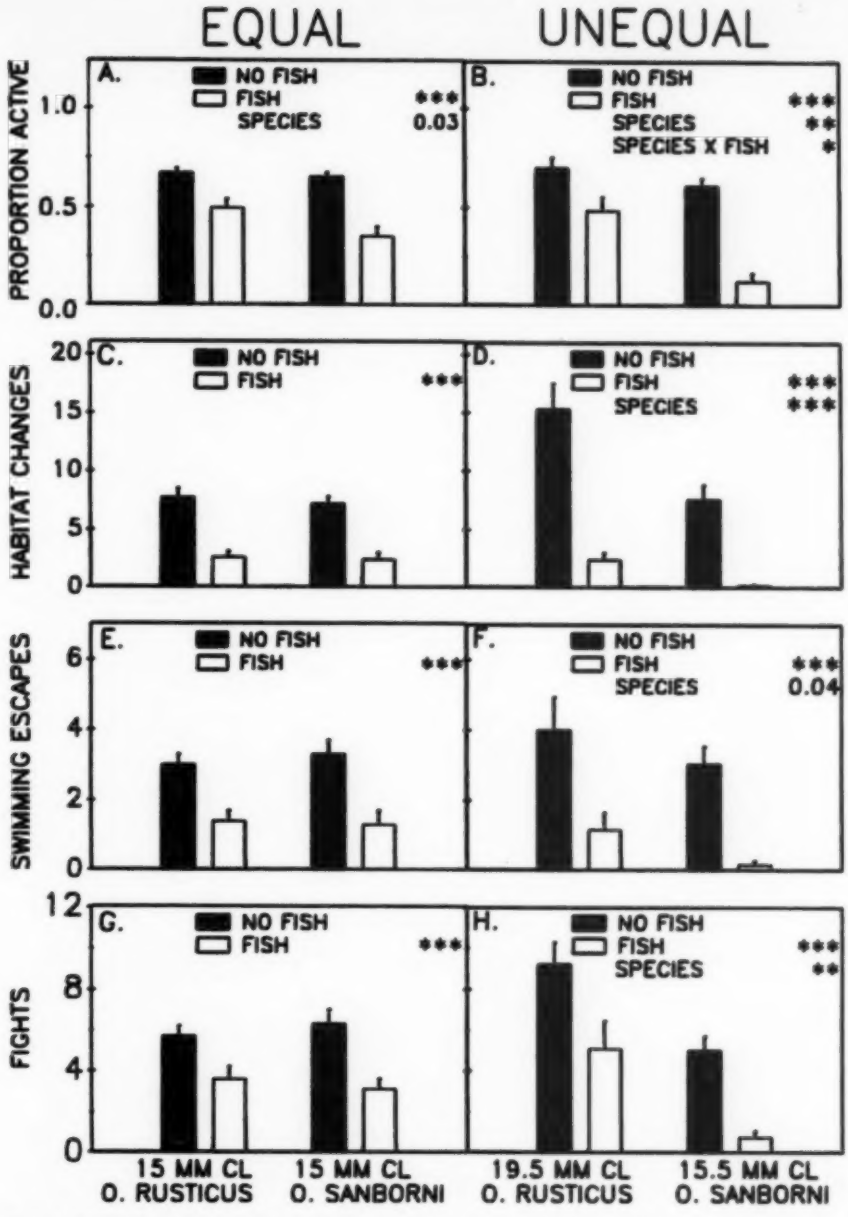

FIG. 4. Effect of fish on (A and B) proportion active, (C and D) habitat changes, (E and F) swimming escapes, and (G and H) fights of *O. rusticus* and *O. sanborni*. In the left-hand panels, both crayfish species were of equal size (N = 7–9); in the right-hand panels, crayfish were of unequal size, reflecting a natural size difference (N = 3). Data are means ± 1 SE. A four-way ANOVA tested for effects of fish predators, crayfish species, adult crayfish, and shelters on juvenile crayfish activities and behaviors. When necessary to stabilize the variance, data were transformed (proportions, arcsin transformation, other data, log transformation). Because five nonlethal responses were measured, trends were considered significant at the $p \leq 0.05/5$ or $p \leq 0.01$ level. NS, $p > 0.01$; *$p \leq 0.01$; **$p \leq 0.001$; ***$p \leq 0.0001$. Significant SS, F, df are given in the text.

unequal size, fish reduced activity of the small, vulnerable *O. sanborni* more than the large, less-vulnerable *O. rusticus* (Fig. 4B: species × fish, SS = 0.44, F = 6.95, df = 47, p = 0.01).

Without fish, equal-sized *O. rusticus* and *O. sanborni* changed habitats with similar frequency (Fig. 4C). However, when sizes reflected a natural size difference, large *O. rusticus* changed habitats more than twice as often as small *O. sanborni*

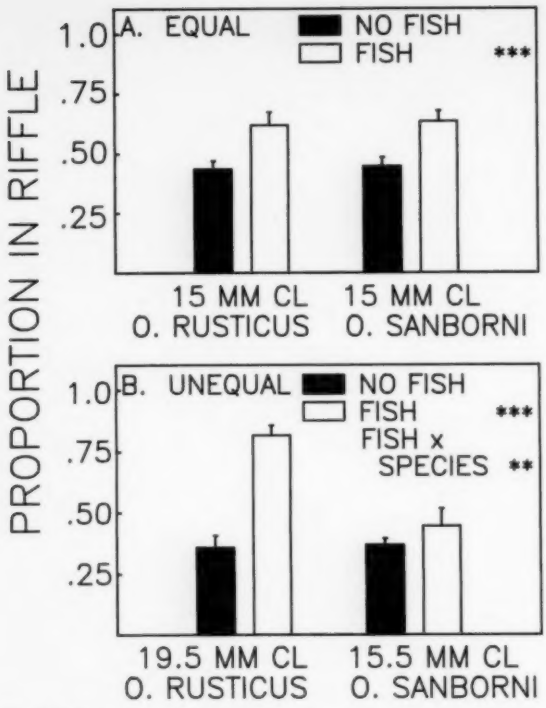

FIG. 5. Effect of fish on habitat use by *O. rusticus* and *O. sanborni*. (A) Both crayfish species were of equal size ($N = 8$); (B) crayfish were of unequal size, reflecting a natural size difference ($N = 3$). Data are means ± 1 SE. A four-way ANOVA tested for effects of fish predators, crayfish species, adult crayfish, and shelters on juvenile crayfish habitat use. When necessary to stabilize the variance, data were arcsin transformed. Because five nonlethal responses were measured, trends were considered significant at the $p \leq 0.05/5$ or $p \leq 0.01$ level. $^{*}p \leq 0.01$; $^{**}p \leq 0.001$; $^{***}p \leq 0.0001$. Significant SS, F, df, and p values are given in the text.

(Fig. 4D: SS = 7.66, $F = 26.68$, df = 47, $p = 0.0001$). With fish, habitat changes declined precipitously for both species and both sizes (Fig. 4C: SS = 786.65, $F = 65.11$, df = 127, $p = 0.0001$; Fig. 4D: SS = 37.41, $F = 130.38$, df = 47, $p = 0.0001$).

Without fish, equal-sized crayfish of both species escaped aggressive threats with a similar swimming response (Fig. 4E). Fish reduced this risky behavior for both crayfish species and sizes (Fig. 4E: SS = 13.70, $F = 38.43$, df = 127, $p = 0.0001$; Fig. 4F: SS = 12.89, $F = 45.24$, df = 47, $p = 0.0001$). Without fish, equal-sized crayfishes fought with similar frequency regardless of species (Fig. 4G). When of unequal size, large *O. rusticus* fought more than small *O. sanborni* (Fig. 4H: SS = 7.36, $F = 17.20$, df = 47, $p = 0.0002$). Fish reduced the number of fights in both species and both sizes (Fig. 4G: SS = 11.76, $F = 25.08$, df = 127, $p = 0.0001$; Fig. 4H: SS = 13.83, $F = 32.32$, df = 47, $p = 0.0001$).

Without fish, equal-sized crayfish did not prefer either habitat (riffles 38%/1 m^2 versus pools 62%/2 m^2; Fig. 5A). With fish, when crayfish were of equal size, both crayfishes increased riffle use and reduced use of dangerous pools (Fig. 5A: SS = 1.05, $F = 18.18$, df = 127, $p = 0.0001$). When crayfish were unequal in size, large, less vulnerable *O. rusticus* increased their use of riffles (82%) and reduced their use of risky pools (18%; Fig. 5B), but small, more vulnerable *O. sanborni* did not shift habitats in response to fish (fish × species, SS = 00.44, $F = 13.56$, df = 47, $p = 0.0008$). Possibly any movement of these small, vulnerable crayfish triggered a lethal attack.

Adult Effects

Adult crayfish did not alter the number of juvenile crayfish eaten for either species or size (Fig. 3). When crayfish were of equal size, *O. rusticus* activities (proportion active and habitat changes) were uninfluenced by adults (Fig. 6A, 6C); *O. sanborni* activity, however showed a biologically suggestive, albeit nonsignificant, increase when adults were present (Fig. 6A: proportion active, adult × species, SS = 0.23, $F = 5.57$, df = 119, $p = 0.02$; Fig. 6C: habitat changes, SS = 61.44, $F = 5.09$, df = 127, $p = 0.03$). The adult effect on activity was in the opposite direction of the fish effect (Fig. 4A and 4C versus Fig. 6A and 6C: fish × adult, SS = 80.55, $F = 6.68$, df = 127, $p = 0.01$).

When crayfish were the same size, adult crayfish dramatically increased swimming escapes for both species (Fig. 6E: SS = 6.41, $F = 17.97$, df = 127, $p = 0.0001$); *O. sanborni* was again more affected by adults than was the invader *O. rusticus* (Fig. 6E: species × adult, SS = 2.20, $F = 6.17$, df = 127, $p = 0.01$). Adults increased the number of fights for both species when crayfish were of equal size (Fig. 6G: SS = 4.93, $F = 10.51$, df = 127, $p = 0.002$). When crayfish were of unequal-size, adult crayfish had no effect on crayfish activity (Fig. 6B, 6D) or behavior (Fig. 6F, 6H), probably because of low sample size and low power ($N = 3$). Neither adults nor shelters affected habitat use for either size or species of crayfish.

Discussion

Direct, Lethal Fish Effects

Both in the field and in our simulated stream, fish predators directly reduced numbers of both crayfishes. In the field survey, when fish were scarce, many factors affect crayfish abundance, and crayfish densities vary widely across sites. But at the few sites where fish were abundant, crayfish density was consistently low, suggesting that fish predators adversely affect crayfish. Although these high-density sites were limited in number, we believe that they demonstrate an important, real trend.

Similarly, predators influence community structure in many systems (Connell 1975), including lakes (Brooks and Dodson 1965; Hall et al. 1970) and ponds (Crowder and Cooper 1982; Morin 1984a, 1984b). In streams, the role of predation is less clear. Some stream predators reduce prey numbers by consumption (Oberndorfer et al. 1984; Power et al. 1985). Others reduce prey numbers only in certain streams (Wilzbach et al. 1986) or of select taxa (Flecker 1984; Walde and Davies 1984). Still other predators have no effect on prey number (Allan 1982; Reice 1983; Flecker and Allan 1984; Reice and Edwards 1986; Culp 1986).

In our view, stream communities where predators have little effect differ predictably from those communities where predators reduce prey number. For example, predator effects are probably unimportant in streams where the following conditions occur: (1) the habitat is highly heterogeneous

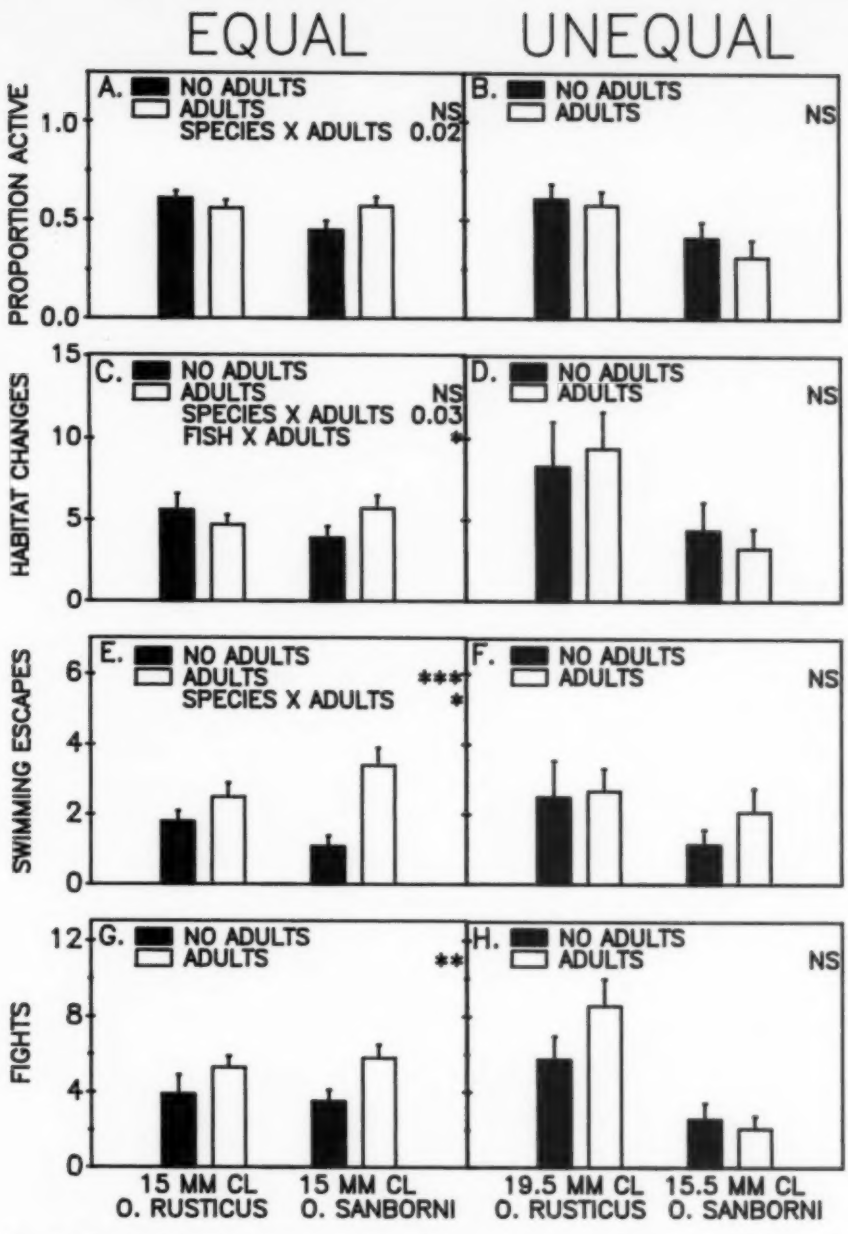

FIG. 6. Effect of adults on (A and B) proportion active, (C and D) habitat changes, (E and F) swimming escapes, and (G and H) fights by *O. rusticus* and *O. sanborni*. In the left-hand panels, both crayfish species were of equal size ($N = 7$–9); in the right-hand panels, crayfish were of unequal size, reflecting a natural size difference ($N = 3$). Data are means ±1 SE. A four-way ANOVA tested for effects of fish predators, crayfish species, adult crayfish, and shelters on juvenile crayfish habitat use. When necessary to stabilize the variance, data were arcsin or log transformed. Because five nonlethal responses were measured, trends were considered significant at the $p \leq 0.05/5$ or $p \leq 0.01$ level. *$p \leq 0.01$; **$p \leq 0.001$; ***$p \leq 0.0001$. Significant SS, F, df, and p values are given in the text.

(Brusven and Rose 1981; Allan 1982, 1983), (2) abiotic conditions are extreme (Peckarsky 1983, 1985; Walde 1986; Schlosser and Ebel 1989), (3) prey immigration rates are high (Flecker and Allan 1984; Cooper et al. 1990), (4) prey demonstrate strong affinity for specific substrate types (Reice 1983; Flecker and Allan 1984), (5) top predators interact weakly with prey (sensu Paine 1980), (6) invertebrate predation compensates for fish predation (Soluk and Collins 1988a, 1988b, 1988c), or (7) where indirect nonlethal effects are not quantified. In our system, we speculate that these conditions

were absent. For example, in our streams, habitat heterogeneity was limited and large-bodied crayfish were relatively unaffected by abiotic disturbances. Crayfish, as active walkers and swimmers, probably drifted infrequently and were not restricted to specific substrates. Crayfish are strongly preferred by smallmouth bass (Stein 1977; Probst et al. 1984; Rabeni 1992), an efficient predator that can consume a wide range of crayfish sizes, and were probably unaffected by invertebrate predators. For these reasons, we speculate that fish predators numerically reduced crayfish prey in our survey and laboratory stream and also contribute to the replacement of *O. sanborni* by *O. rusticus* in Ohio streams.

Indirect, Nonlethal Fish Effects

Predator-induced habitat shifts and other nonlethal, indirect effects also influence prey survival, growth, and reproduction. In our experiments, smallmouth bass reduced crayfish activity, aggressive behaviors, and pool use. In another survey exploring this replacement, crayfish at sites with fish predators were smaller and less dense than at sites without fish, suggesting that predators also influence crayfish growth and habitat use in the field (Mather and Stein 1993). Similar indirect predator effects have been widely reported. For example, fish predators alter the distribution and activities of other stream prey (Stein and Magnuson 1976; Peckarsky 1980; Fraser and Cerri 1982; Sih 1982; Collins et al. 1983; Cooper 1984; Dill and Fraser 1984; Power 1984; Power et al. 1985; Schlosser 1987). Indirect, nonlethal predator effects or a combination of direct and indirect effects appear to be more common in streams than prey reductions due to direct consumption alone (Peckarsky and Dodson 1980; Petranka 1983; Hildrew et al. 1984; Power et al. 1985; Bowlby and Roff 1986; Feltmate and Williams 1989). Whether this is an inherent quality of stream systems is unclear. For the Ohio replacement, clearly both direct and indirect effects were important.

In our work, juvenile activity was inconsistently related to survival. Whereas increased activity has negative consequences, i.e., attracts predators, increased activity also may have positive consequences, i.e., lead to increased feeding, opportunities to change habitats, and advantages in competitive encounters. When crayfish were small, increased activity probably consistently increased predation risk, but when crayfish were larger and able to repel initial fish attacks, increased activity, habitat changes, fights, and swimming escapes probably provided crayfish with a chance to escape attack. Hence, size plays an important part in determining how increased crayfish activity affects survival.

Among other crayfish, size-related aggression can cause differential feeding (Lorman 1975) and habitat use (Butler and Stein 1985; Rabeni 1985). In our experiments, responses of juveniles to adults were counter to those elicited by fish; thus, the presence of adults could further increase predation risk for juvenile crayfish. Our work with field patterns and individual behavioral responses supports the hypothesis that size-mediated predation contributes to the replacement. Our experiments show that (1) fish prefer small over large crayfish, (2) fish reduce activity and frequency of all behaviors of small crayfish more than large crayfish, and (3) adult crayfish may exacerbate the predator–prey interaction. Thus, through a variety of mechanisms, size affects this replacement. Because the displaced native *O. sanborni* is smaller than the invader, fish predators may differentially reduce both survival and growth of *O. sanborni*.

Shelter Effects

In other systems, habitat heterogeneity modifies interactions between predators and prey by providing refuges (Stein 1977; Brusven and Rose 1981; Savino and Stein 1982; Holomuzki 1989) or by affecting dispersal (Huffaker 1958). Predators can also influence substrate choice (Feltmate et al. 1986); conversely, substrate can alter patterns of selective predation (Peckarsky and Penton 1989). Surprisingly, shelters in our study did not modify juvenile crayfish survival, activity, or behavior. Apparently, crayfish had other behavioral options available; reducing activity, flattening against the substrate, shifting into riffles, and using shelters were all common responses. If shelterless when a fish appeared, these responses appeared to be more appropriate than shelter-seeking. Because *O. rusticus* and *O. sanborni* did not differ in response to shelters, habitat heterogeneity per se probably does not contribute to the replacement. However, if refuges are limited in streams, being small could be especially risky. Because *O. sanborni* is smaller than *O. rusticus*, under conditions of shelter limitation, the native, again, might be disadvantaged.

Species Replacement

Abiotic Factors

No evidence exists that abiotic factors influence replacement rates (Capelli and Magnuson 1983; Flynn and Hobbs 1984). Neither in Wisconsin (Capelli 1982; Capelli and Magnuson 1983) nor in Ohio (Mather 1990) do lakes and streams containing *O. rusticus* appear to differ abiotically from adjacent environments without the invader. Therefore, we believe that abiotic factors are less important than biotic ones.

Species

Species-specific differences in competitive ability exist among orconectid crayfish (Bovbjerg 1970). Equal-sized *O. rusticus* are innately more aggressive than the native crayfish *O. virilis* in northern Wisconsin (Capelli and Munjal 1982). Unlike this Wisconsin replacement, in Ohio, innate species differences are less important than body size (Butler and Stein 1985). Our work further demonstrates that *O. sanborni* and *O. rusticus* differ little except that *O. rusticus* (1) is less susceptible to adult aggression, (2) is larger, and (3) tends to be more active. Because *O. sanborni* is more similar to *O. rusticus* than the displaced Wisconsin species, mechanisms underlying the Ohio replacement are, we think, more subtle. In turn, these similarities may explain why the replacement is occurring relatively slowly in Ohio streams.

Size

For other animals, as size increases, predation risk declines (Werner and Gilliam 1984; Semlitsch and Gibbons 1988). Similarly, big crayfish are eaten less frequently than small crayfish. Crayfish reach an invulnerable size (measured as TL) at about 20% of fish predator length (Stein 1977); in this study, large *O. rusticus* 19.5 mm CL were 15% of predator length and approaching the size refuge. Hence, body size has a critical impact on the outcome of crayfish interactions, especially predator–prey relationships.

Size had at least three other effects. First, without fish, big crayfish shifted habitat, swam, and fought more than small crayfish. Where these activities confer an advantage, large

crayfish should benefit. Second, as in other size-structured communities (Sih 1982; see Sih 1987 for a more complete review), more vulnerable, small *O. sanborni* reduced activity more in response to the fish threat than the less vulnerable, large *O. rusticus*. If reduced activity reduces long-term growth, then *O. sanborni* should suffer disproportionately. Third, in the equal-size experiments, both species shifted into riffles with fish; however, when of unequal size, only the large juveniles shifted, suggesting that interactions between large and small juveniles prevented riffle use by small *O. sanborni*. Because large nonadults also can affect small juveniles negatively, rapid growth should always be favored for crayfish and larger size should be an advantage to *O. rusticus*.

Relationships among prey species, size, activity, and predation rates are complex. We examined individual behaviors and assumed that these reponses would translate into population-level effects. However, crayfish population dynamics are not well understood and compensatory mechanisms could affect these populations. To test our conclusions, population responses of the two species should be examined explicitly.

Rates of Replacement

Because the exact time of each introduction of *O. rusticus* is unknown, rates of replacement in the field and how fish, adults, and shelters influence these rates are unknown. However, based on data herein, we postulate that fish predators accelerate this crayfish species replacement and that adult crayfish affect the replacement by exacerbating this predator–prey interaction. Thus, both indirect and direct effects of predation, mediated through size, play an important role in the replacement of *O. sanborni* by *O. rusticus* in Ohio streams.

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Changes in Prey Fish Populations in Western Lake Erie, 1969–88, as Related to Walleye, *Stizostedion vitreum*, Predation

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Relative abundance of the total prey fish community in the western basin of Lake Erie varied little from 1969 to 1988, but species composition changed significantly. Soft-rayed fishes such as emerald shiner, *Notropis atherinoides*, spottail shiner, *N. hudsonius*, and alewife, *Alosa pseudoharengus*, declined significantly after 1977 whereas only one spiny-rayed species, white bass, *Morone chrysops*, declined over the same period. Trout-perch, *Percopsis omiscomaycus*, a relatively abundant species rarely eaten by piscivores in this system, experienced only minor shifts in abundance between 1969 and 1988. Although several factors could be responsible for the shift in species composition, predation by increasingly abundant walleye, *Stizostedion vitreum*, played a major role. Walleye prefer to eat soft-rayed fishes; thus, observed shifts in the community match expectations of selection noted in the diet. We suggest that management goals focusing primarily on walleye affected not only the targeted species but the entire fish community of western Lake Erie.

L'abondance relative de la communauté totale de poissons proies dans le bassin occidental du Lac Érié a peu varié de 1969 à 1988, mais la composition spécifique a changé de façon notable. Les poissons à nageoires rayonnées come le méné émeraude, *Notropis atherinoides*, la queue à tache noire, *N. hudsonius*, et le gaspareau, *Alosa pseudoharengus*, ont nettement décliné après 1977, tandis qu'une seule espèce à nageoires épineuses, le bar blanc, *Morone chrysops*, marquait un recul pendant la même période. L'omisco, *Percopsis omiscomaycus*, espèce relativement abondante mais rarement consommée par les piscivores dans ce système, n'a présenté que des modifications mineures de l'abondance entre 1969 et 1988. Si les changements observés dans la composition spécifique peuvent être imputables à plusieurs facteurs, la prédation due à une population de plus en plus abondante de doré, *Stizostedion vitreum*, a joué un rôle de premier plan. Le doré préfère se nourrir de poissons à nageoires rayonnées, et les changements observés dans la communauté correspondent aux prévisions que permet le choix alimentaire de cette espèce. Nous pensons que les buts de la gestion, qui ont été axés avant tout sur le doré, ont affecté non seulement l'espèce visée, mais l'ensemble de la communauté ichtyologique de l'ouest du Lac Érié.

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Lake Erie provides a natural setting to evaluate the role of selective piscivory on a multispecies prey fish community in a large system. Walleye, *Stizostedion vitreum*, are the dominant piscivores in Lake Erie and their population characteristics have received considerable attention historically (Regier et al. 1969; Wolfert 1969; Parsons 1970, 1971; Kutkuhn et al. 1976; Muth and Wolfert 1986; Hatch et al. 1987). Walleye abundance declined during the 1960's, remained low through the mid-1970's, but increased rapidly after 1976 due to conservative fishery management and the production of strong year classes in 1977, 1982, and 1986 (Hatch et al. 1987; Ohio Department of Natural Resources, unpublished data). In the mid-1980's, walleye in the western basin may have exceeded historical levels and expanded into the central basin of Lake Erie and other connecting waters.

The substantial increase in predator abundance prompted concerns regarding system carrying capacity (Knight et al. 1984; Muth and Wolfert 1986). Walleye prefer soft-rayed fishes over spiny-rayed fishes in Lake Erie and are size selective, eating mostly small (<130 mm total length) prey even as adults (Knight et al. 1984). Because these predators depend on annual production of age-0 fishes in Lake Erie (Knight et al. 1984; Hartman and Margraf 1992), walleye predation could alter the fish species composition and size structure of the community. Although such shifts might be attributed to other factors such as a reduction in the rate of eutrophication (e.g., Leach et al. 1977) or the presence of an exotic species (e.g., white perch, *Morone americana*), effects due to predation should be magnified in the presence of abundant walleye and when viewed over a short time interval.

We examined differences in fish community structure between an earlier period (1969–77) of low walleye abundance in western Lake Erie and a later one (1978–88) of high walleye abundance to ascertain the effecs of piscivory. Prey fish abun-

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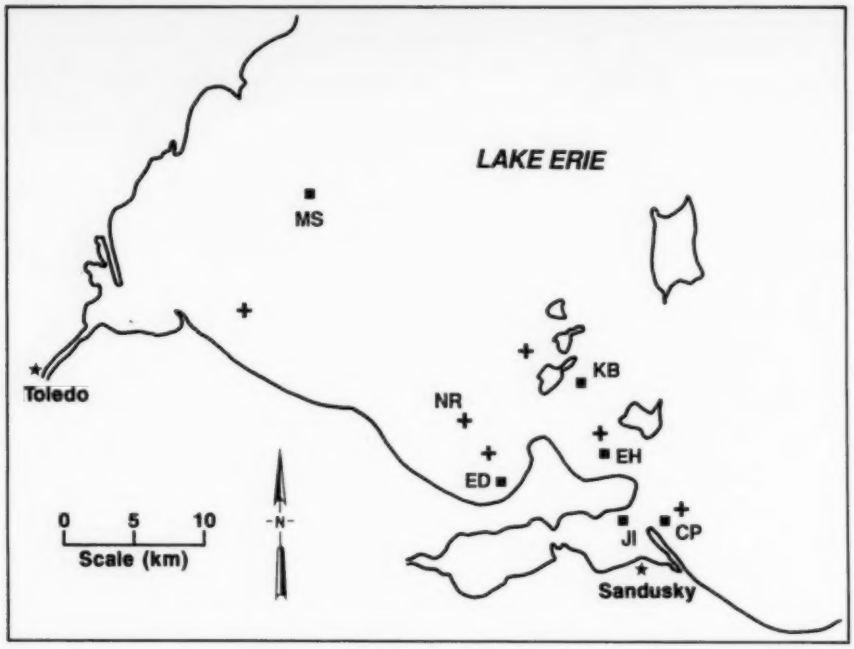

FIG. 1. Sampling stations for ODNR bottom trawl surveys in western Lake Erie, 1969–88. Offshore index stations are designated at MS and KB, nearshore stations at ED, EH, JI, and CP, and supplemental stations as "+" (NR = Niagara Reef).

TABLE 1. Physical characteristics of western Lake Erie waters measured by ODNR during trawling surveys, 1969–88.

	n[a]				Mean water temperature (°C)[b]				Mean dissolved oxygen ($mg \cdot L^{-1}$)[b]				Median Secchi transparency (m)[c]			
Year	June	July	Aug.	Oct.	June	July	Aug.	Oct.	June	July	Aug.	Oct.	June	July	Aug.	Oct.
1969	3	6	7	2	18.0	23.5	25.1	19.5+	—	—	—	—	0.6	0.8	1.2	—
1970	12	12	18	0	21.9	23.2	24.7	—	9.6	8.3	7.9	—	0.6	0.8	0.6	—
1971	11	12	12	5	23.4	23.7	23.7	20.2	8.1	8.5	8.4	8.6	0.6	0.8	0.8	—
1972	12	12	12	5	20.8+	23.8+	22.2+	14.8+	10.3+	7.5+	8.2+	9.4	0.6	0.8	0.9	—
1973	3	5	2	7	21.0+	24.2+	25.0+	17.0+	10.0+	—	9.5+	10.0	0.6	0.6	—	—
1974	6	15	13	7	17.5+	24.0+	25.7+	14.0+	—	8.1+	8.0+	—	0.8	0.9	0.8	0.8
1975	10	14	12	7	22.2+	24.4+	23.4	14.4	—	—	—	10.0	0.8	1.2	0.9	0.9
1976	10	21	12	7	21.2	23.9	21.0	13.7	9.6	10.2	—	10.9	0.9	0.8	0.9	—
1977	12	8	13	2	19.5	23.8	24.1	10.5	11.0	11.2	10.1	—	0.6	0.9	0.9	0.9
1978	6	11	10	3	23.0	22.9	24.0	12.3	—	—	—	—	0.8	0.9	0.9	—
1979	6	14	9	6	20.2	22.4	22.5	13.2+	—	—	—	—	0.3	0.6	0.9	—
1980	11	9	6	2	19.3	23.3	19.8	—	—	—	—	—	0.3	0.9	0.8	0.6
1981	6	0	0	0	21.7	—	—	—	—	—	—	—	—	—	—	—
1982	18	16	18	7	19.3	23.4	21.3	17.9	8.5	7.8	8.6	9.3	0.6	0.9	0.9	—
1983	16	16	16	6	20.9	23.9	25.8	13.5	8.2	6.0	7.1	8.8	0.9	1.1	0.9	1.1
1984	16	16	16	6	19.8	21.8	23.3	15.7	7.9	7.7	6.7	9.0	0.6	0.8	1.1	0.3
1085	17	16	9	6	19.5	22.6	22.7	14.3	8.4	7.6	7.2	9.5	0.6	0.9	0.9	1.1
1986	21	14	18	7	21.5	24.7	24.6	13.4	7.5	6.6	7.5	8.9	0.6	1.2	0.6	0.6
1987	14	16	6	6	21.6	24.5	25.0	17.3	7.7	6.1	7.0	9.0	1.2	0.9	0.9	1.4
1988	8	11	8	6	21.9	24.5	22.4	18.8	8.3	6.6	5.0	8.3	0.9	1.1	0.9	0.8

[a]Number of stations where at least one variable was measured.
[b]Unmarked data are from bottom waters; marked data (+) are from surface waters.
[c]Nearshore stations only.

dance data from bottom trawl assessment surveys in conjunction with previous studies of walleye diets were used to measure these changes. The early period was established as the time prior to the recruitment of the strong 1977 walleye year class to the yearling–adult population whereas the later period reflected substantial increases in walleye abundance (Hatch et al. 1987). Our objectives were to examine annual abundance and species composition of prey fishes and prey composition

of the walleye diet in relation to the change in walleye abundance between these periods.

Materials and Methods

Study Area

Physical and limnological characteristics of the western basin of Lake Erie are well documented (Beeton 1963; Hartman 1972; Sly 1976; Boyce et al. 1987). Generally, the western basin is isothermal throughout the year due to mixing of its shallow waters and seldom becomes anoxic. Bottom substrates consist primarily of sand and clay, although dolomite limestone forms several major reef complexes. Stations were established in the western basin at several soft-bottomed areas (Fig. 1) amenable to trawling and were assumed to be representative of prey fish populations throughout the western basin, despite the lack of samples from reef habitats and from waters outside of Ohio's jurisdiction. Abiotic data collected during this study are summarized in Table 1.

Prey Fish Field Collections

Fish population data were collected biweekly from June through August (summer) and in October (fall) by the Ohio Department of Natural Resources (ODNR) at four nearshore (2–7 m depth) and two offshore (>9 m depth) index stations in the western basin of Lake Erie from 1969 to 1988 (Fig. 1). Fishes were collected with a semiballoon trawl (10.7-m headrope, 6.4-mm bar mesh in cod end) towed on the lake bottom at about 0.8 $m \cdot s^{-1}$. Nearshore stations were sampled for 10 min and offshore stations were sampled for 15 min to provide adequate catches (catches generally were lower in offshore areas during summer). Paired tows were made in opposite directions at each station but were not considered independent samples because of overlapping tow swaths; thus, total effort was 20 min at nearshore stations and 30 min at offshore stations.

Summer surveys were designed to quantify relative abundance of age-0 walleye and yellow perch, *Perca flavescens*. Surveys began soon after age-0 walleye were first captured in preliminary tows in early June. Supplemental stations (Fig. 1) were established at offshore (depth >6 m) areas after 1977 to provide additional data on fish distribution patterns.

Fall trawl surveys consisted of single visits to the sampling stations (Fig. 1). Fall surveys began by early October and were completed within 3 wk. As in summer, supplemental stations were sampled after 1977 to provide additional data on fish distribution.

Catches were sorted and counted by species (Table 2) and size group aboard ship. If abundance of any species/size group was high (exceeding approximately 1000 fish), total numbers were estimated from random subsamples obtained either volumetrically (±0.5 L) or gravimetrically (±30 g) from the total volume or weight of that group. Rainbow smelt, *Osmerus mordax*, were not always counted in summer surveys; hence, we included them in relative abundance analyses for fall only.

Prey Fish Data Analyses

To estimate relative abundance, we first converted catch per tow to catch per hour of trawling (CPHT) at each index station. These data then were transformed as ln(CPHT + 1) to approximate a normal distribution (Sokal and Rohlf 1986) and to serve as a relative abundance index.

TABLE 2. Common and scientific names of fishes captured during bottom trawl surveys in western Lake Erie, 1969–88. Species regularly captured at every station are identified with a "+". Prey of walleye are identified with a "p".

	Common name	Scientific name
+, p	Alewife	*Alosa pseudoharengus*
	Bigmouth buffalo	*Ictiobus cyprinellus*
	Black bullhead	*Ameiurus melas*
	Black crappie	*Poxomis nigromaculatus*
	Blackside darter	*Percina maculata*
	Bluegill	*Lepomis macrochirus*
	Bluntnose minnow	*Pimephales notatus*
	Bowfin	*Amia calva*
+	Brown bullhead	*Ameiurus nebulosus*
	Burbot	*Lota lota*
+	Channel catfish	*Ictalurus punctatus*
	Coho salmon	*Oncorhynchus kisutch*
+	Common carp	*Cyprinus carpio*
+, p	Emerald shiner	*Notropis atherinoides*
	Fathead minnow	*Pimephales promelas*
+, p	Freshwater drum	*Aplodinotus grunniens*
+, p	Gizzard shad	*Dorosoma cepedianum*
	Golden shiner	*Notemigonus crysoleucas*
	Goldfish	*Carassius auratus*
	Northern hog sucker	*Hypentelium nigricans*
	Johnny darter	*Etheostoma nigrum*
	Lake whitefish	*Coregonus clupeaformis*
	Largemouth bass	*Micropterus salmoides*
	Longnose gar	*Lepisosteus osseus*
	Logperch	*Percina caprodes*
	Mooneye	*Hiodon tergisus*
	Muskellunge	*Esox masquinongy*
	Northern pike	*Esox lucius*
	Pumpkinseed	*Lepomis gibbosus*
+	Quillback	*Carpiodes cyprinus*
+, p	Rainbow smelt	*Osmerus mordax*
	Rainbow trout	*Oncorhynchus mykiss*
	Rock bass	*Ambloplites rupestris*
	Sand shiner	*Notropis stramineus*
	Sauger	*Stizostedion canadense*
	Sea lamprey	*Petromyzon marinus*
	Shorthead redhorse	*Moxostoma macrolepidotum*
	Silver chub	*Macrhybopsis storeriana*
	Silver lamprey	*Ichthyomyzon unicuspis*
	Smallmouth bass	*Micropterus dolomieu*
	Spotfin shiner	*Cyprinella spiloptera*
+, p	Spottail shiner	*Notropis hudsonius*
	Stonecat	*Noturus flavus*
+, p	Trout-perch	*Percopsis omiscomaycus*
+, p	Walleye	*Stizostedion vitreum*
+, p	White bass	*Morone chrysops*
	White crappie	*Pomoxis annularis*
+, p	White perch	*Morone americana*
	White sucker	*Catostomus commersoni*
+, p	Yellow perch	*Perca flavescens*

Index values were calculated in two ways: as the arithmetic mean of the log-transformed CPHT across four nearshore stations (pooled species) in summer and as the mean transformed CPHT across offshore stations for each species in fall. Seasonal selection of near and offshore stations for index calculations was based on apparent offshore movements of many species in late summer and fall. Pooling of species into prey type categories (discussed below) was performed on CPHT values prior to transformation.

TABLE 3. Relative abundance statistics for all fish species caught in ODNR summer bottom trawl surveys in western Lake Erie, 1969–88.

	June			July			August		
Year	n[a]	Mean[b]	95% CI[c]	n	Mean	95% CI	n	Mean	95% CI
1969	4	1171.6	183, 7471	4	3747.1	420, 33356	4	1217.0	4, 18209
1970	8	781.9	217, 2800	8	3618.2	1082, 12090	12	2127.1	925, 4890
1971	6	83.7	19, 343	8	2701.7	388, 18749	8	2454.3	782, 7694
1972	7	177.0	27, 1129	8	520.7	115, 2336	8	4039.0	633, 25739
1973	4	135.0	30, 595	12	4998.0	1590, 15701	8	5983.9	1663, 21514
1974	4	278.5	6, 9960	11	2685.5	459, 15686	9	12518.0	3002, 52177
1975	6	1174.0	135, 10132	10	1129.0	247, 5132	8	4410.6	1864, 10434
1976	7	1297.5	404, 4162	8	11463.4	6346, 20707	8	11474.8	5917, 22252
1977	8	1261.7	226, 6997	8	2848.8	228, 35373	8	4203.9	946, 18656
1988	4	1338.4	56, 30965	8	4632.2	1251, 17134	8	3525.3	918, 13543
1979	4	146.2	8, 2294	8	1661.4	605, 4552	8	26956.0	15425, 41015
1980	7	400.8	101, 1581	8	8777.0	2306, 33397	4	13133.5	8019, 21508
1981	8	661.5	65, 6553	8	8094.0	917, 71310	8	8005.4	3085, 20766
1982	12	2015.3	1059, 3834	8	8945.3	3464, 23094	8	3046.3	1849, 5019
1983	8	198.7	89, 440	8	1890.3	905, 3946	8	2789.6	1032, 7534
1984	8	320.2	84, 1213	8	8750.7	3536, 21650	8	20069.4	7959, 50600
1985	8	613.6	145, 2570	8	1698.3	436, 6604	4	9995.6	520, 191548
1986	8	378.2	105, 1346	8	4933.5	1212, 20059	8	13959.7	7458, 26127
1987	7	153.6	34, 670	8	3669.2	1458, 9231	4	2696.3	737, 9848
1988	4	3546.5	896, 14025	8	18470.8	8604, 39651	4	43782.0	9595, 199748
Pooled									
1969–77	54	506.2	306, 837	77	2739.8	1703, 4407	73	4397.4	3040, 6361
1978–88	78	530.1	356, 788	88	4973.1	3571, 6925	72	8475.1	6197, 11591

[a]Number of trawl stations.
[b]Geometric mean of catch per hour of trawling.
[c]Back-transformed lower and upper limits of 95% CI.

Summer index values were used to monitor monthly changes in prey fish abundance and were assumed to directly reflect annual production. We estimated species composition of trawl catches during August (generally the month of highest prey fish availability) as the mean percentage of the CPHT among stations. Fall index values were assumed to reflect prey fish abundance after most of the annual predation by walleye had occurred (Hartman and Margraf 1992).

We designated "prey fishes" to include major species/size groups known to be vulnerable to walleye during the year (Knight et al. 1984). Prey fishes included all sizes of most soft-rayed fishes (e.g., emerald shiner, *Notropis atherinoides*, spottail shiner, *N. hudsonius*, rainbow smelt, and trout-perch, *Percopsis omiscomaycus*) but only age-0 clupeids (gizzard shad, *Dorosoma cepedianum*, and alewife, *Alosa pseudoharengus*). Spiny-rayed fishes were considered vulnerable only as age 0 and included walleye, white bass, *Morone chrysops*, white perch, yellow perch, and freshwater drum, *Aplodinotus grunniens*. Age-1 and older clupeid and spiny-rayed fishes were not vulnerable to most walleye because they generally exceed 130 mm (total length) in summer and fall (Knight et al. 1984; ODNR, unpublished data). Age-0 walleye also were too large (>200 mm) to be prey in fall and were excluded from analyses.

Species were grouped into clupeid, soft-, and spiny-rayed prey type categories to account for differences due to food selection by walleye (Knight et al. 1984). Clupeids were separated from other soft-rayed fishes because of their frequent consumption by walleye and because their fast growth allows them to outgrow vulnerability to most walleye. Other soft-rayed fishes remain at vulnerable sizes to walleye throughout their lives. Spiny-rayed fishes were separated from both clupeids and other soft-rayed fishes because of their fast growth and low consumption by walleye.

Walleye Food Habits

A review of walleye food habits literature produced four sources of data from which we could determine seasonal composition of the three prey types to the diets of age-0 and age-1 or older walleye in western Lake Erie (Wolfert 1969; Parsons 1971; Knight 1982; Hartman and Margraf 1992). Published data were averaged (when necessary) across stations and/or ages (1 and older) to provide monthly composition percentages by prey type, year, and walleye age group (0 or older) from each study. Seasonal diet composition was measured for each walleye age group as the average and range of monthly percentages across spring (April–June), summer (July–August), and fall (September–November) of each year. Percentages were based on numbers (Parsons 1971), volumes (Wolfert 1969; Knight 1982), or wet weight (Hartman and Margraf 1992).

Results

Prey Fish Abundance and Size

Relative abundance (all species pooled) was similar between 1969–77 and 1978–88 for all summer months. In June, average total catch rates were low in both periods, probably because larval and early juvenile fishes were not fully vulnerable to the trawl. Total catches increased significantly (t-tests, $P < 0.05$) between June and July–August and typically were highest in August (Table 3; Fig. 2). However, differences in total catch rates were not significant (t-tests, $P > 0.05$) between periods

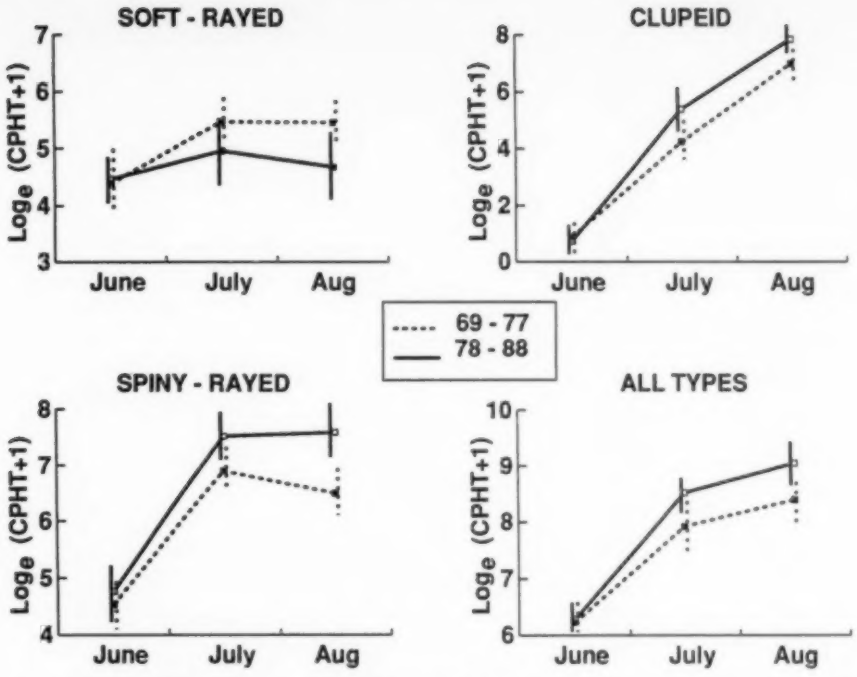

FIG. 2. Mean log-transformed catch per hour trawled (CPHT) with 95% confidence intervals for three types of prey fishes from ODNR bottom trawl catches at nearshore stations during summer, 1969–88.

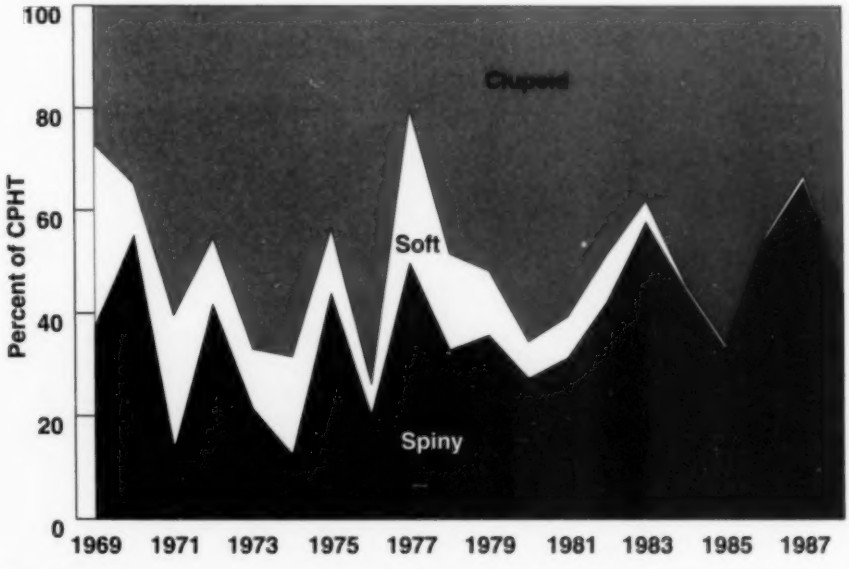

FIG. 3. Mean percent contribution to total catch per hour trawled (CPHT) for three prey fish types collected from ODNR bottom trawl surveys at nearshore stations in western Lake Erie during August, 1969–88.

for any month. Likewise, differences in mean index values were apparent for each of the various prey types between periods but were significant ($P < 0.05$) only for spiny-rayed fishes in August (Fig. 2), which became more abundant during 1978–88.

Shifts in species composition were apparent between periods during August trawl surveys, consisting mostly of a decrease in the proportion of soft-rayed fishes and an increase in the proportion of spiny-rayed fishes (mostly white perch) after 1977 (Fig. 3). Gizzard shad and alewife fluctuated widely over the

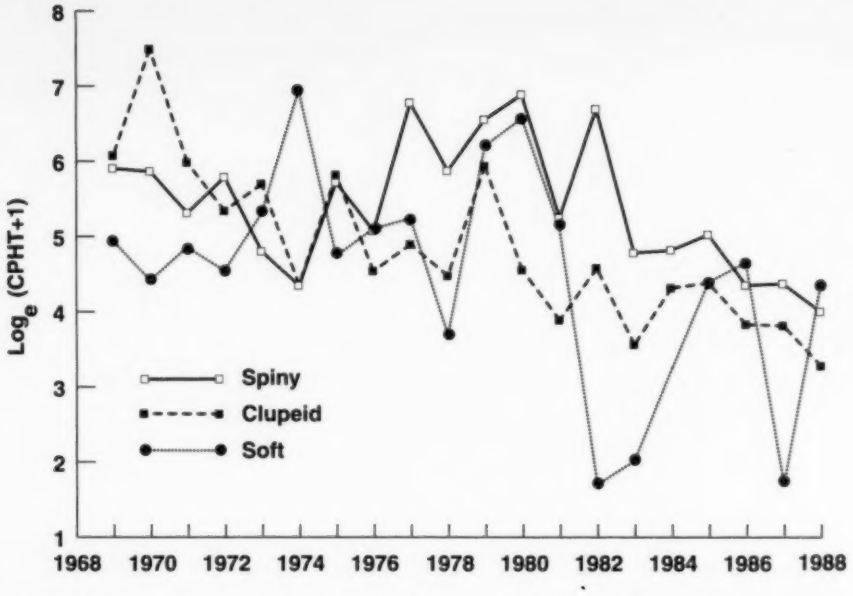

FIG. 4. Relative abundance trends for three prey fish types during fall ODNR bottom trawl surveys at two offshore stations in western Lake Erie, 1969–88. Index values were calculated as the mean log-transformed catch per hour trawled (CPHT) across both stations for each year.

TABLE 4. Geometric mean of catch per hour of trawling for fishes caught in ODNR fall bottom trawl surveys at two offshore stations in western Lake Erie, 1969–88.

	Soft-rayed species[a]						Spiny-rayed species[b]				
Year	AW	GS	ES	SS	TP	RS	WB	WP[c]	YP	WA	FD
1969	162.2	56.0	994.6	351.6	4.9	40.6	112.9	—	163.7	0.0	607.5
1970	26.7	26.7	1564.3	56.2	43.3	25.9	59.2	—	1153.0	0.7	12.5
1971	37.0	12.1	434.5	176.2	59.2	41.8	5.9	—	625.4	0.0	213.6
1972	18.0	2.6	289.1	60.3	0.7	9.2	14.9	—	506.2	0.0	119.9
1973	95.3	12.1	103.8	244.2	12.0	1.8	0.0	—	43.9	0.7	17.7
1974	731.2	20.8	189.4	56.6	14.2	157.6	14.2	—	92.3	4.0	91.3
1975	20.1	4.0	172.4	60.7	17.9	19.7	19.6	—	692.0	46.5	186.7
1976	193.4	11.1	102.6	181.0	45.9	41.7	27.3	—	101.3	6.1	159.9
1977	25.5	13.3	39.8	170.9	198.5	56.1	8.0	0.0	1512.2	8.7	132.0
1978	5.7	53.7	20.2	253.9	35.2	2.6	2.3	0.0	329.3	14.0	345.9
1979	41.8	60.1	2.6	236.9	312.2	312.2	4.6	0.7	1877.1	13.0	184.9
1980	179.4	231.5	7.7	93.1	13.9	14.3	14.2	0.0	1799.8	20.3	24.2
1981	9.8	274.1	0.0	6.5	86.0	0.0	0.7	7.5	294.6	3.2	26.2
1982	0.0	3.4	4.2	5.1	156.4	11.2	2.6	48.7	1301.4	22.5	18.4
1983	1.6	3.6	14.6	17.1	72.5	3.6	0.0	182.3	15.5	0.7	139.2
1984	8.4	2.3	0.0	15.6	62.0	36.5	7.8	2017.3	246.4	7.8	30.8
1985	1.2	156.9	0.0	1.2	73.7	0.0	1.2	252.9	55.0	0.0	54.8
1986	10.1	140.2	3.1	6.4	73.2	37.3	5.7	250.9	35.1	42.7	22.8
1987	2.0	4.9	4.9	7.3	83.6	24.1	0.7	1019.5	28.5	3.6	87.9
1988	4.6	27.5	0.7	6.9	56.1	20.7	1.2	884.4	30.5	0.7	58.7
Pooled[d]											
1969–77	65.3	12.8	241.2	121.0	20.4	27.2	14.7	0.0	309.9	2.3	87.4
1978–88	6.7	32.2	2.8	17.5	71.4	11.0	2.5	54.6	168.4	6.1	136.4

[a]AW = alewife, GS = gizzard shad, ES = emerald shiner, SS = spottail shiner, TP = trout-perch, RS = rainbow smelt.
[b]WB = white bass, WP = white perch, YP = yellow perch, WA = walleye, FD = freshwater drum.
[c]White perch were not observed in trawl catches until summer 1977.
[d]Geometric mean per period (N = 18 observations during 1969–77 and 22 during 1978–88).

TABLE 5. Percent composition of three prey fish types to the seasonal diets of walleye of age 0 and ≥1 in western Lake Erie during 1959–60 (Parsons 1971), 1962 (Wolfert 1969), 1979–81 (Knight 1982), and 1986–88 (Hartman and Margraf 1992).

Season	Age	Year	n^a	Prey fish type: Clupeids Mean (range)	Soft-rayed Mean (range)	Spiny-rayed Mean (range)
Spring	≥1	1960	2	0.0 (0–0)	100.0 (100–100)	0.0 (0–0)
(Apr.–June)		1979–81	7	1.8 (0–16)	91.4 (78–100)	6.8 (0–18)
		1986–88	3	8.2 (4–15)	46.1 (12–70)	45.7 (26–83)
Summer	0	1959	2	1.0 (0–2)	61.5 (50–73)	38.0 (26–50)
(July–Aug.)		1962	5	31.0 (0–86)	12.5 (0–50)	56.5 (7–100)
		1979–81	2	48.3 (40–57)	37.2 (34–40)	14.5 (9–20)
		1986–88	6	22.0 (2–47)	9.7 (3–22)	68.3 (45–95)
	≥1	1960	2	32.5 (5–60)	44.0 (0–88)	23.5 (7–40)
		1979–81	6	75.0 (69–100)	6.7 (0–20)	18.3 (0–80)
		1986–88	6	66.4 (27–96)	8.6 (0–38)	25.0 (3–64)
Fall	0	1959	2	0.0 (0–0)	88.0 (76–100)	12.0 (0–24)
(Sept.–Nov.)		1962	3	9.4 (0–28)	67.9 (11–100)	22.7 (0–61)
		1979–81	3	18.2 (5–33)	70.8 (50–95)	11.0 (0–33)
		1986–88	4	25.0 (6–60)	43.5 (12–76)	31.5 (12–46)
	≥1	1960	2	84.0 (68–100)	16.0 (0–32)	0.0 (0–0)
		1979–81	8	55.6 (0–95)	37.2 (5–100)	7.2 (0–49)
		1986–88	8	93.9 (83–97)	4.4 (0–18)	1.7 (0–3)

[a]Number of monthly percent values used in the calculation of mean and range.

20-yr period but generally composed a relatively constant fraction of trawl catches over the long-term trend. Alewife was the predominant clupeid in the early period, but gizzard shad became increasingly abundant after the mid-1970's. After 1977, age-0 gizzard shad and white perch composed more than 85% of August trawl catches.

In fall, substantial declines in relative abundance were evident after 1977 for alewife, emerald shiner, and white bass (Table 4). Spottail shiner declined after 1980 and yellow perch after 1982. Gizzard shad and trout-perch increased in relative abundance after 1977. As a group, spiny-rayed fishes maintained relatively constant densities over the 20-yr period, with some decline occurring after 1982 (Fig. 4). Clupeids, on average, were significantly less abundant (t-tests, $P < 0.05$) in the later period compared with the earlier period. Declines for other soft-rayed fishes during 1982–88 were the most substantial of any prey type.

Mean index values in fall declined significantly (t-tests, $P < 0.05$) between 1969–77 and 1978–88 for alewife, both shiner species, and white bass and were lower (although not significantly so) for all species except gizzard shad, trout-perch, freshwater drum, and walleye during the latter period (Fig. 5). For emerald shiner, this decline began early in the 20-yr period concurrent with the increase in the walleye population (Table 4). In contrast, spottail shiner maintained constant abundance until the early 1980's before they declined. Overall, relative abundance for four of six soft-rayed and two of four spiny-rayed fishes decreased in the period of high walleye abundance, which accounted for a significant ($P < 0.05$) decline in total fall catch rates (all species) relative to the early period of low walleye abundance.

Walleye Food Habits

Eleven of the 15 most commonly encountered fishes in trawl surveys were eaten by walleye (Table 2) (Knight et al. 1984; Hartman and Margraf 1992). Walleye diets varied among years (studies) within seasons and age groups (Table 5). In spring, age-1 and older walleye ate mostly shiner in 1960 and during 1979–81, but spiny-rayed prey were more prevalent during 1986–88. In summer, age-0 walleye usually ate a higher proportion of spiny-rayed prey than did older walleye, which ate mostly clupeids or shiner in all years. There was evidence of higher shiner consumption during the summers of 1959–60 than in later years by both walleye age groups. Fall diets of age-0 walleye were dominated by shiner in all years whereas older walleye ate mostly clupeids. Spiny-rayed fishes were not the major prey of age-1 and older walleye in summer or fall from any period. Generally, walleye ate more shiner in early years than in later years and more spiny-rayed prey in later years, except for age-1 and older walleye, which ate mostly clupeids in summer and fall in all years.

Discussion

Walleye and Community Structure

We contend that predation by abundant walleye, the terminal piscivores in this system, played a large role in structuring the fish community from 1969 to 1988. Our conclusion is based on observed changes in annual prey abundance and species composition in western Lake Erie as walleye abundance increased over this period (Hatch et al. 1987). Walleye in Lake Erie are known to be selective for prey type (Knight et al. 1984; Francis 1992), exhibiting positive electivity values for most soft-rayed fishes (excluding trout-perch) and negative values for spiny-rayed prey. In this study, we show that significant declines in relative abundance occurred primarily for soft-rayed fishes (particularly shiner) but not for spiny-rayed fishes or trout-perch between 1969–77 and 1978–88.

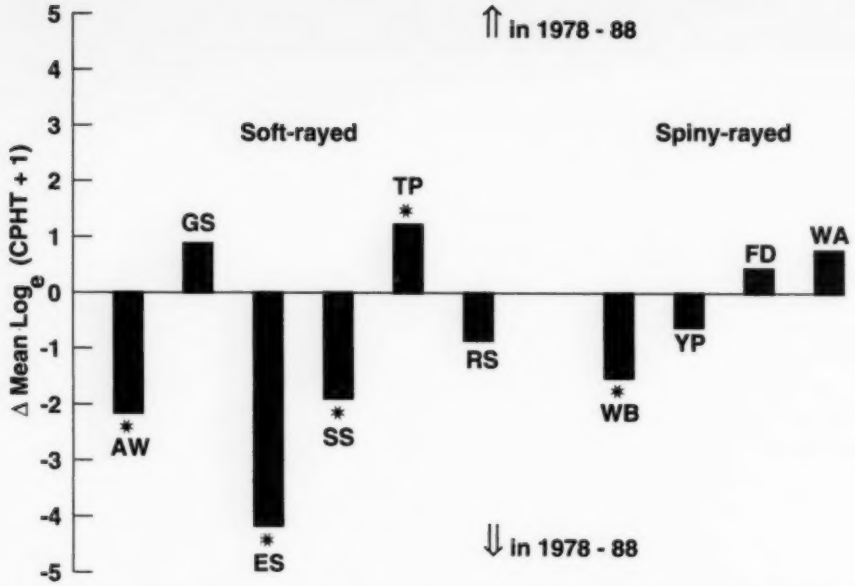

FIG. 5. Differences in mean log-transformed catch per hour trawled (CPHT) between 1978–88 and 1969–77 for six soft-rayed and four spiny-rayed prey fishes from fall ODNR botom trawl catches at offshore stations in western Lake Erie. Significant differences (*t*-tests, $P < 0.05$) are indicated with an asterisk. Species codes are defined in Table 4.

Implicit in our conclusion is the assumption that walleye feeding behavior did not change substantially between periods. A review of walleye diet studies in western Lake Erie produced no information from 1969 to 1977 but did provide data from 1959 to 1962 (Wolfert 1969; Parsons 1971) when walleye abundance was similarly low (Hatch et al. 1987) and from 1979 to 1988 (Knight 1982; Hartman and Margraf 1992) when their abundance was high. Diet shifts between 1959–62 and 1979–88 reflect major changes in the composition of the prey fish community between periods but not necessarily changes in walleye feeding behavior. For example, increased use of spiny prey in spring by walleye during 1986–88 (Hartman and Margraf 1992) coincided with high abundance of white perch and low abundance of shiner. However, walleye did not become entirely opportunistic, as soft-rayed prey were the prevalent item in stomachs despite their low abundance. Changes in summer and fall diets across periods also coincided with changes in abundance of soft-rayed prey, and walleye consistently switched to clupeids as opposed to spiny prey. Francis (1992) concluded that diet selection of Lake Erie walleye during 1990–91 was determined primarily by prey type as opposed to abundance, soft-rayed prey being selected over spiny prey. Therefore, we believe that walleye feeding behavior was consistent between 1959 and 1988 and, consequently, their selective predation on soft-rayed prey had a major influence on fish community structure.

Structuring of aquatic communities by predation has been well documented. Numerous studies on planktivore–plankton interactions have demonstrated community shifts in size structure and species composition as a result of size-selective predation (see Northcote 1988 for an excellent review). The effects of piscivory on prey fish populations also have received considerable attention. Using regression analyses on published data, McQueen et al. (1986) determined that although food availability to any trophic level establishes the maximum level of attainable biomass, piscivore effects on planktivore biomass are strong in both oligotrophic and eutrophic systems. Robinson and Tonn (1989) concluded that piscivory was at least as important as abiotic factors in structuring fish communities of Alberta (Canada) lakes.

Although most field studies involving piscivory have occurred in small systems, there are examples in large systems as well. Mills and Forney (1988) summarized that nutrient/food availability regulates production at each trophic level but that piscivorous walleye control biological structure of the fish community in Oneida Lake. In Lake Michigan, Stewart et al. (1981) concluded that introduced salmonids consumed 20–30% of the annual alewife production, which in turn had major ramifications on the structure of an extensive planktivorous fish community. To our knowledge, no other studies exist concerning the effects of piscivory on the prey fish community in Lake Erie, but Makarewicz and Bertram (1991) attributed recent increases in the number of large-bodied cladocerans in Lake Erie to top-down control of planktivorous fishes by walleye.

Undoubtedly, some shifts in the Lake Erie fish community were caused by factors other than walleye predation. Changes in nutrient load (Leach et al. 1977; Hayward and Margraf 1987), loss of spawning habitat (Regier et al. 1969), fisheries exploitation (Hartman 1972), and interactions with exotic species (Schneider and Leach 1979) have been suggested as stressors on percid communities of the Great Lakes (Edwards and Ryder 1990).

Loading rates of phosphorus into Lake Erie peaked in the late 1960's (Sly 1976) and then declined (Rathke and McRae 1989). This reduction likely promoted recovery of the depressed

walleye population, as may have occurred in the Bay of Quinte (Hurley 1986), but effects on other members of the fish community are unknown. Annual prey fish production was similar from 1969 to 1988, as measured by summer index values. Reductions in nutrient loading should have lowered biomass at all trophic levels and increased the effect of top-down control (McQueen et al. 1986). Consistent production suggests that reductions in phosphorus loadings had not yet caused substantial declines in energy to secondary trophic levels, perhaps due simply to lagged responses. Because only age-0 abundance was measured for most species, however, it is possible that production shifted from older to younger age groups during 1978–88 relative to 1969–77 and masked any real differences in abundance between periods. If this did occur, it is plausible that walleye predation allowed more energy to be shunted to reproduction because of reduced intraspecific competition.

Effects of fisheries exploitation were substantial for percids historically (Parsons 1970; Hartman 1972; Hatch et al. 1987) but were negligible on adult forage fishes (e.g., gizzard shad), which have limited commercial or recreational value in Lake Erie. A small-scale commercial bait industry has existed on Lake Erie for several decades, primarily targeting on shiner, but harvests are believed to be insignificant relative to total population sizes (G. Emond, ODNR, personal communication).

With the exception of white perch, no exotic species flourished in the western basin from 1969 to 1988. Lake Erie white perch are zooplanktivorous at age 0 but become opportunistic as adults, eating fish when availability is high (Schaeffer and Margraf 1986; Parrish 1988; Parrish and Margraf 1990, 1991; Prout et al. 1990). Because of their feeding habits, white perch may influence fish community structure both through competition and predation. Hartman et al. (1992) examined diet overlap among several planktivorous fish species in Lake Erie and concluded that some level of competition occurred between white perch and native fishes. Studies in both Lake Erie (Parrish and Margraf 1990, 1991) and Oneida Lake (Prout et al. 1990) indicate high potential for competition between age-0 white perch and yellow perch. The effects of white perch as piscivores are unknown. We believe that white perch had a role in the declining population trends of many prey fishes (e.g., white bass, yellow perch, spottail shiner, emerald shiner) in Lake Erie after 1982, but had little effect prior to then due to ther own low abundance (Boileau 1985).

Reductions of emerald and spottail shiner population in recent years have major ramifications on community stability. During years of low gizzard shad production, the availability of small (<100 mm) prey fishes during winter and spring may be limiting to juvenile walleye and other piscivores. Although spiny-rayed fishes have become more abundant in recent years, they are more difficult for small piscivores to capture and handle than are soft-rayed prey (Weithman and Anderson 1977; Gillen et al. 1981; Wahl and Stein 1990). Additionally, reductions in shiner populations may have increased food availability to other planktivores, such as gizzard shad and white perch, because shiner feed primarily on large zooplankton (Muth and Busch 1989; Hartman et al. 1992).

Management Implications

In western Lake Erie, interagency fisheries management traditionally has been directed at percid species. By implementing conservative management actions to sustain walleye rehabilitation in Lake Erie, fishery managers probably have influenced fish community structure via the "top-down" effects of walleye predation. Hartman and Margraf (1992) suggested that current management techniques used by Lake Erie agencies are unlikely to affect walleye biomass when strong year classes are produced; hence, effects of predation on the fish community are simply endured. Additionally, several consecutive year-class failures may be necessary to reduce piscivory sufficiently to increase populations of prey fish (Stewart et al. 1981; Carpenter et al. 1985). Thus, continued single-species management to maintain high walleye abundance may result in unstable prey fish populations.

Shuter (1990) proposed that small, short-lived fish species are useful indicators of community stress. Significant reductions of key prey species (e.g., emerald and spottail shiner) may serve as an appropriate indicator that carrying capacity was exceeded. A loss of forage species diversity may equate to a loss in community stability (Elton 1958), particularly if remaining forage fishes are less preferred by predators and/or experience highly variable annual recruitment that translates into a variable food supply to walleye. Reductions in native fishes also may allow exotic species to become established or flourish.

Biological control of exotic fishes in Lake Erie by abundant walleye, as suggested by Hurley and Christie (1977) in the Bay of Quinte, may not be a realistic expectation for spiny-rayed invaders. Despite increased use of age-0 white perch by walleye in Lake Erie (Hartman and Margraf 1992), recruitment of white perch continued to increase throughout the 1980's. Even if walleyes contribute to significant overwinter mortality of age-0 white perch, competitive interactions between white perch and native species are probably most intense as age-0 individuals (Prout et al. 1990) and will have already occurred. In contrast, recruitment of soft-rayed exotics (alewife and rainbow smelt) in the western basin declined during 1978–88. We predict that of future exotic fishes introduced into Lake Erie, only spiny-rayed fishes will proliferate in pelagic areas. Soft-rayed fishes are unlikely to become established in the presence of intense walleye predation.

In summary, we provide evidence that walleye are influencing fish community structure in Lake Erie. It is evident from this study and from the literature that management of a top predator can indirectly affect the structure of the entire fish community, even in systems as large as Lake Erie. Hopefully, future studies will quantify prey supply (as suggested by Ney 1990), as well as further substantiate predator demand (e.g., Hartman and Margraf 1992) in Lake Erie, so that limits to carrying capacity can be included in risk analyses of management strategies available to resource managers. This should greatly assist agencies in establishing community as opposed to single-species management goals.

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Environmental Sex Determination in Southern Brook Lamprey, *Ichthyomyzon gagei*

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Sex ratios were determined for 20 populations of southern brook lamprey larvae, *Ichthyomyzon gagei*, collected from throughout much of their range in the southeastern United States. Ratios varied between 9 and 49% males. Differential mortality was an unlikely factor, as sex ratios were similar among age groups within each population. Environmental sex determination is suggested. Sex ratio varied with growth (expressed as length of larvae at specific ages), larval density, pH, and annual mean temperature of the natal stream. Generally, under conditions promoting rapid growth, the percentage of males varied directly with density and inversely with temperature. Where growth was slow, the percentage of males declined as larval density increased, the response being less at low than at high pH. Temperature had little effect when larval growth was slow. The percentage of males declined when growth was rapid under otherwise similar environmental conditions.

Nous avons calculé le rapport des sexes dans 20 populations de lamproie, *Ichthyomyzon gagei*, à l'état larvaire, recueillies dans une bonne partie de leur aire de répartition du sud des États-Unis. Les rapports variaient de 9 à 49 % de mâles. La différence dans le taux de mortalité semble un facteur peu probable, car les rapports des sexes étaient similaires entre les groupes d'âge dans chaque population. On peut proposer que le sexe est déterminé par l'environnement. Le rapport des sexes variait en fonction de la croissance (exprimée par la longueur des larves à des âges spécifiques) de la densité des larves, du pH et de la température annuelle moyenne du cours d'eau natal. En général, dans des conditions favorisant une croissance rapide, le pourcentage de mâles variait en fonction directe de la densité et en fonction inverse de la température. Quand la croissance était lente, le pourcentage de mâles baissait à mesure que la densité de larves augmentait, la réaction étant moins forte à un pH bas qu'à un pH haut. La température avait peu d'effet quand la croissance des larves était lente. Le pourcentage de mâles baissait quand la croissance était rapide dans des conditions environnementales par ailleurs similaires.

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The phylogenetically primitive lamprey (Bardack and Zangerl 1971; Hardisty 1979) are characterized by a long larval period which is spent within the soft substrate of cool-water streams. For much of the larval period the gonads remain undifferentiated (Okkelberg 1921; Hardisty 1965a, 1965b). Parasitic lamprey, those species that have retained the juvenile period during which they feed on the body fluids and tissues of fishes, mature a large number of oocytes (Vladykov 1951; Beamish and Potter 1975). Nonparasitic or brook lamprey have not retained the juvenile period and are less fecund (Vladykov 1951; Abakumov 1966; Beamish and Thomas 1983) than their presumed parasitic ancestors (Hubbs 1925; Hubbs and Trautman 1937; Zanandrea 1959; Potter 1980).

The potential for exponential increase in abundance of the parasitic sea lamprey, *Petromyzon marinus*, was realized soon after their invasion of the upper Great Lakes in the late 1930s (Applegate 1950; Smith and Tibbles 1980). A characteristic of this period of increasing abundance was a preponderance of males in adult populations (Smith 1971). Sea lamprey abundance was reduced precipitously following treatment with the lampricide 3-trifluoromethyl-4-nitrophenol (TFM), and the proportion of male larvae and adults correspondingly declined (Smith 1971; Purvis 1979). Since there is no evidence to suggest that TFM is differentially toxic to male and female lamprey (Manion and Smith 1978), it appears that sex differentiation in sea lamprey may be influenced by population size.

There is little doubt that sex has a genetic basis among the lower vertebrates despite the frequent absence of recognizable sex chromosomes (Yamamoto 1953; Richards and Nace 1978; Hunter et al. 1983; Yamazaki 1983). In at least some taxa among the lower vertebrates, the expression of sex is both variable and labile (Atz 1964; Bull 1980; Yamazaki 1983). Environmental conditions are known to irreversibly alter primary sex differentiation in some gonochoristic species. Egg incubation temperature (Bull and Vogt 1979; Conover and Kynard 1981; Ferguson and Joanen 1982; Mohanty-Hejmadi and Dimond 1986), pH (Rubin 1985), and population density (D'Ancona 1950; Lindsey 1962) have been identified as factors causing environmental sex determination in some taxa of lower vertebrates. Kirpichnikov (1981) suggested that male and female genes in fish are located in many chromosomes and the determination of sex depends on the balance of these genes. Each gene affecting gonad development is thought to be relatively weak in action, and therefore, a change in environmental conditions and the resulting variation in genotype may easily be accompanied by changes in population sex ratio.

Environmental sex determination has been related to population density in least brook lamprey, *Lampetra aepyptera*, with

the proportion of males increasing directly with the species' abundance (M.F. Docker and F.W.H. Beamish, Department of Zoology, University of Guelph, Guelph, Ont., personal observation). The present study examined 20 populations of southern brook lamprey, *Ichthyomyzon gagei*, from much of their geographical distribution (Lee et al. 1980) for evidence of environmental sex determination.

Materials and Methods

Southern brook lamprey were electrofished from streams in which it was the only species of lamprey. Identification was confirmed from diagnostic taxonomic characteristics displayed by adults collected from each stream (Hubbs and Trautman 1937). Some streams were sampled for lamprey only once, and others several times over a period of years (Table 1). Generally, lamprey were collected from more than one location in a stream or, when access was extremely difficult, from a length of stream in excess of 1 km. The area of substrate electrofished was measured to allow calculation of larval density.

Several broad-spectrum indicators of water quality were usually measured each time a stream was sampled. Total hardness (EDTA) and alkalinity (pH 4.5) were measured directly in the field (APHA 1989). Conductivity and pH were measured, also in the field, with probes that were regularly calibrated. Annual mean temperatures were estimated from temperature records for these or nearby streams (U.S. Department of Commerce, National Technical Information Service, Water Resources Data for Alabama, Arkansas, Louisiana, Mississippi, and Texas, water year 1987).

Immediately after capture, all animals were either anaesthetized in tricaine methanesulfonate (1% MS222 by weight) or killed by an overdose of the anaesthetic and total length measured to the nearest millimetre. This information was used to construct length–frequency distributions as one method of estimating population age structure. Larvae that were killed were frozen for subsequent determination of sex and removal of statoliths, structures analogous to teleost otoliths and similarly used for age determination (Beamish and Medland 1988).

In the laboratory, frozen lamprey were thawed, again measured for total length, statoliths removed, and the gonads examined. Lengths of thawed larvae were adjusted to correspond to live length on the basis of the following regressions:
For larvae 25–75 mm in thawed length:

$$L_l = 1.04L_t - 0.95 \qquad (n = 150)$$

For larvae 76–200 mm in thawed length:

$$L_l = 1.10L_t - 3.50 \qquad (n = 150)$$

where L_l is total length of live larvae and L_t is total length of thawed larvae. All lengths in this study are reported as live total length.

Statoliths were removed from the otic capsules of larvae and stored in immersion oil for 10–30 d to intensify the banding patterns (Medland and Beamish 1987). Each statolith was examined under a dissecting microscope and, when annuli were recognizable, aged independently two or three times. Statoliths were numerically coded so that the estimation of age was not biased by the length of that individual or by an age previously assigned. The same age was almost always assigned on repeated examination of the same statolith. Where it was not possible to age individuals, a length–frequency distribution was constructed for the population by taking a moving average over 7 mm to enhance the frequency peaks (Hardisty 1961) and the age of individuals assigned on the basis of their length.

TABLE 1. Locations of streams from which southern brook lamprey were captured and collection dates. When only a single collection was made from a stream, the month is given.

State	County or parish	Stream	Date
Alabama	Tuscaloosa	Binion Cr.	1987–90
Alabama	Macon	Choclafaula Cr.	1980–91
Alabama	Covington	Eden Cr.	1989–90
Alabama	Covington	Teel Cr.	1989–90
Alabama	Escambia	Hell Hole Cr.	1987–91
Arkansas	Hot Springs	Keisler Cr.	April 1989
Arkansas	Hot Springs	Thomas Cr.	April 1989
Arkansas	Saline	South Fork Saline R.	April 1989
Arkansas	Saline	Ten Mile Cr.	April 1989
Louisiana	Tangipahoa	Beaver Cr.	April 1990
Louisiana	Tangipahoa	Spring Cr.	April 1989
Louisiana	Tangipahoa	Terry's Cr.	April 1989
Louisiana	Grant	Big Cr.	April 1990
Louisiana	Grant	Clear Cr.	April 1989
Louisiana	Grant	Dry Prong Cr.	April 1989
Louisiana	Grant	Dyson Cr.	April 1990
Mississippi	Simpson	Uspoha Cr.	April 1987
Mississippi	Perry	Water Prong Cr.	1987–90
Texas	Nacagdoches	Legg Cr.	April 1989
Texas	Tyler	Little Cypress Cr.	April 1989

Sex of the larger larvae was determined by visual internal inspection of the gonad under a dissection microscope. Histological examinations were conducted on all of the smaller larvae and, periodically, on the larger lamprey to confirm the identification made by visual inspection. Histological examinations were based on two 5-mm-thick cross-sections taken from the midregion of an individual, fixed in 5% formalin for a minimum of 48 h, and embedded in parafin. The subsequent 8-μm-thick sections were stained with hematoxylin and eosin (Willey 1971) and sex was determined under a compound microscope (Docker 1992).

Results

Southern brook lamprey spawn in the spring (Raney 1952; Dendy and Scott 1953; Beamish 1982). Observations on the population in Choclafaula Creek, Alabama, over an 11-yr period indicate that spawning commences early in April and is complete within about 4 wk. Other populations in Alabama have been observed to follow a similar spawning schedule. In Louisiana, spawning activity among lamprey from the seven streams sampled was most intense during mid-April. Spawning appears to reach a peak during early to mid-April in Mississippi and is complete by late April. Southern brook lamprey spawn about a week earlier than this in those streams examined in Arkansas and Texas. In general, spawning activity appears to be most intense during mid-April throughout the geographic region examined in this study. It is probable that eggs hatch within 2–3 wk after spawning (Piavis 1971). For the purpose of this study, May 1 was assigned as the southern brook lamprey's birthdate in all streams.

The assignment of age from annuli on statoliths was possible only for the population of brook lamprey from Binion Creek. In other populations, annuli were not recognizable and age was assigned from length–frequency distributions. For a sample of

TABLE 2. Relationship between total length and age in southern brook lamprey larvae. In the linear growth equation, L is modal total length (millimetres) of larvae and A is age (months). All correlation coefficients (r) were significant at $P < 0.05$ for the sample sizes, (n) used to derive the equations.

Stream	Total no. of larvae	Growth equation $L = a + bA$			
		a	b	n	r
Binion Cr.	211	55.5	2.1	8	0.990
Choclafaula Cr.	4742	22.3	2.7	34	0.998
Eden Cr.	239	40.6	3.1	8	0.983
Teel Cr.	174	23.3	2.3	4	0.991
Hell Hole Cr.	611	49.7	1.9	12	0.999
Keisler Cr.	155	5.6	2.6	4	0.996
Thomas Cr.	153	20.4	2.4	4	0.999
South Fork Saline R.	76	8.8	2.8	4	0.987
Ten Mile Cr.	217	39.5	2.5	4	0.998
Beaver Cr.	73	55.1	2.6	4	0.989
Spring Cr.	91	59.8	2.3	4	0.984
Terry's Cr.	138	39.8	2.7	4	0.985
Big Cr.	152	12.7	3.2	4	0.998
Clear Cr.	189	18.5	2.5	4	0.999
Dry Prong Cr.	83	6.5	3.0	4	0.997
Dyson Cr.	131	31.2	2.3	4	0.997
Uspoha Cr.	269	23.1	2.2	4	0.999
Water Prong Cr.	260	21.0	2.0	4	0.998
Legg Cr.	171	24.4	2.4	4	0.999
Little Cypress Cr.	181	27.4	2.4	4	0.997

larvae collected from Binion Creek in late April 1988, mean total length (±SD) of larvae aged 24, 36, and 48 mo, on the basis of statolith banding patterns, was 79.0 ± 20.2 (n = 7), 110.5 ± 12.6 (n = 26), and 134.7 ± 9.2 mm (n = 22). These were similar to the modal lengths of 74, 101, and 132 mm estimated from the length–frequency distribution for the same collection of larvae from Binion Creek.

A growth equation was derived for larvae from each stream or population based on modal lengths and age. For most populations, it was not possible to compare growth among age classes. However, with the extensive data base for Choclafaula Creek, length throughout larval life was compared among six successive age classes beginning in 1976 (SAS 1989; general linear model). Lengths for each age group (i.e., 12, 24, 36 mo) were not significantly different among the six age classes ($F_{5,21}$ = 1.00, $P > 0.05$). Hence, all modal lengths for larvae of a given age from Choclafaula Creek were pooled and a single growth equation derived:

$$L = 22.3 + 2.7A \qquad (n = 34; r = 0.990)$$

where L is live total length (millimetres) of larvae and A is age (months).

For each of the other populations, one to three collections of lamprey were made. Length–frequency curves indicated a larval life of 4.25 yr (51 mo) for most animals in all streams, with the probability that a small number of animals complete the larval interval 1 yr earlier. Modal lengths from the length–frequency distributions were used to derive growth equations (Table 2) which were used to calculate lengths at 12, 24, 36, and 48 mo.

Sex of southern brook lamprey could be determined for most larvae older than 11 mo. Prior to sex differentiation, gonads contained only small numbers of undifferentiated germ cells. The ovary of the differentiated female was characterized by the appearance of a number of nucleated basophilic oocytes (Docker 1992). Males could be recognized only by the absence of oocytes, since differentiation of germ cells into spermatocytes does not occur in larvae.

Sex ratio varied among populations from 9 to 49% males (Table 3). Sex ratio was not significantly different from parity ($P > 0.05$) in five of the populations. In the remaining populations, females were significantly the more abundant sex. Generally, the overall bias in sex ratio for a population was maintained across all age groups and was especially evident where numbers were large (Fig. 1). In most populations, mean length of females in the oldest age group was greater than that of males. However, differences were significant only in Teel and Keisler creeks and in one collection from each of Choclafaula and Water Prong creeks (t-statistic; $P < 0.05$). Among the younger age groups, there was neither statistical support nor a pattern of difference in length between the sexes.

The percentage of males within a population was not related to larval density ($P > 0.05$) but varied inversely with modal total length at a given age, the latter being a measure of growth. Modal total lengths were calculated from the equations in Table 2. The relationship between percentage of males and length-at-age among populations was linear and significant ($P < 0.05$) and is described by the following equations:

$$S_m = 80.3 - 0.6L_2 \qquad (n = 20; r = 0.663)$$
$$S_m = 88.2 - 0.5L_3 \qquad (n = 20; r = 0.554)$$
$$S_m = 87.6 - 0.4L_4 \qquad (n = 18; r = 0.531)$$

where S_m is the percentage of male larvae within a population and L_2, L_3, and L_4 are the modal total lengths of larvae at 24, 36, and 48 mo, respectively.

The range in concentration of total hardness, alkalinity, and conductivity within and among streams was not large (Table 4), and for each identity, it was not possible to demonstrate a significant relationship with the percentage of males. Similarly, the percentage of males was not significantly correlated with the annual mean temperature estimated for each of the 20

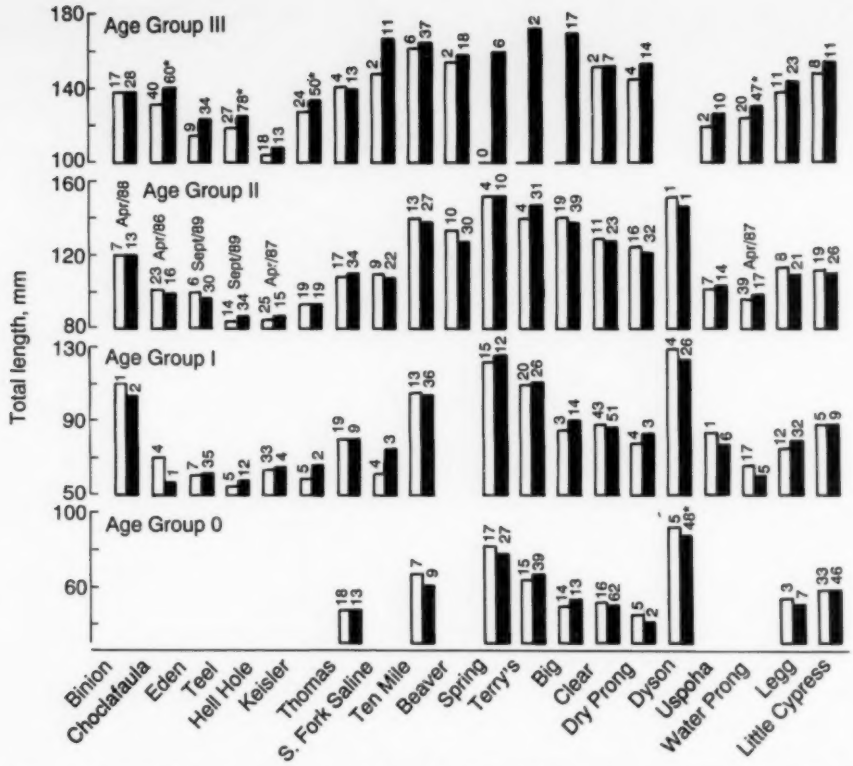

FIG. 1. Mean total length of male (open bars) and female (solid bars) larvae by age group. Sample size is indicated by the number above each bar. Significant differences in length ($P < 0.05$) between sexes within an age group are indicated with an asterisk. Two or more collections of larvae were made from each of the streams in Alabama and from Water Prong Creek in Mississippi (Table 1), but the results of only one representative collection are reported in the figure. Sampling dates for the other streams are as reported in Table 1.

TABLE 3. Sex ratios of southern brook lamprey populations and density of larvae based on surface area of substrate electrofished. Significant deviations in sex ratio from parity, based on 95% confidence limits (CL) for proportions (Beyer 1968), are identified with asterisks.

Stream	No. of larvae sexed	Sex ratio		Larval density (no.·m^{-2})
		% ♂	95% CL	
Binion Cr.	120	37*	28–45	0.17
Choclafaula Cr.	1707	41*	35–47	0.68
Eden Cr.	121	19*	12–27	0.29
Teel Cr.	170	27*	26–35	0.13
Hell Hole Cr.	219	49	44–57	1.75
Keisler Cr.	119	40*	32–48	0.04
Thomas Cr.	127	46	37–55	0.18
South Fork Saline R.	51	29*	17–43	1.90
Ten Mile Cr.	148	26*	19–34	1.55
Beaver Cr.	60	20*	11–32	0.26
Spring Cr.	91	40	30–51	—
Terry's Cr.	137	28*	21–37	—
Big Cr.	119	30*	22–39	1.52
Clear Cr.	185	39*	32–47	1.20
Dry Prong Cr.	80	36*	27–47	0.99
Dyson Cr.	95	9*	4–17	0.50
Uspoha Cr.	40	38	23–54	—
Water Prong Cr.	219	45	40–53	1.13
Legg Cr.	117	29*	21–39	0.24
Little Cypress Cr.	157	41*	33–49	0.23

TABLE 4. Physicochemical characteristics (mean ± SD) of the streams from which southern brook lamprey were captured. n = sample size.

Stream	Annual mean temperature (°C)	n	pH	Conductivity (μS)	Alkalinity ($mg \cdot L^{-1}$)	Total hardness ($mg \cdot L^{-1}$)
Binion Cr.	20.9	5	7.1 ± 0.1	22 ± 5	10 ± 4	12 ± 5
Choclafaula Cr.	14.6	14	7.2 ± 0.2	127 ± 18	115 ± 11	97 ± 9
Eden Cr.	20.9	4	7.0 ± 0.3	68 ± 14	34 ± 4	34 ± 4
Teel Cr.	20.9	4	7.2 ± 0.2	94 ± 22	47 ± 10	50 ± 11
Hell Hole Cr.	19.1	7	6.8 ± 0.2	21 ± 3	10 ± 3	13 ± 4
Keisler Cr.	20.0	3	6.3 ± 0.2	39 ± 10	9 ± 2	12 ± 3
Thomas Cr.	15.5	3	6.4 ± 0.4	37 ± 9	13 ± 2	14 ± 3
South Fork Saline R.	15.5	2	7.1	134	44	51
Ten Mile Cr.	19.1	3	7.2 ± 0.2	86 ± 24	28 ± 5	35 ± 10
Beaver Cr.	17.5	2	6.5	43	15	16
Spring Cr.	17.5	3	6.6 ± 0	47 ± 4	17 ± 9	16 ± 2
Terry's Cr.	20.0	4	6.8 ± 0.1	40 ± 4	16 ± 6	13 ± 3
Big Cr.	17.5	3	7.0 ± 0.2	46 ± 2	14 ± 4	12 ± 1
Clear Cr.	20.0	3	7.1 ± 0.3	47 ± 8	22 ± 5	12 ± 3
Dry Prong Cr.	20.0	1	6.2	32	18	12
Dyson Cr.	20.0	2	6.8	33	15	12
Uspoha Cr.	17.9	4	6.6 ± 0.2	36 ± 8	17 ± 3	13 ± 3
Water Prong Cr.	17.9	3	6.0 ± 0	21 ± 8	9 ± 2	9 ± 2
Legg Cr.	18.4	2	7.1	113	34	35
Little Cypress Cr.	18.1	2	7.0	87	27	37

streams. Percentage of male lamprey decreased as pH increased between 6.0 and 7.2. However, the correlation between percentage of males and pH was not significant ($P > 0.05$). When the sex ratio for larvae from Beaver Creek was excluded, on the assumption a bias may have occurred due to the small sample ($n = 60$), the regression was significant ($P < 0.05$):

$$S_m = 116.3 - 12.1p \qquad (r = 0.443)$$

where p represents pH.

The combined effects of larval growth and density and stream temperature and chemistry on sex ratio were examined by multiple regression analysis (SAS 1989; REG procedure) and expressed by the regression

$$S_m = 175.5 - 2.4L_2 + 0.7L_2 \cdot D + 0.3L_2 \cdot p - 3D \cdot p + 5.2TD - 0.8 \cdot 10^{-1} L_2 \cdot T$$

where L_2 is the total length of larvae at 24 mo, T is the annual mean stream temperatures (degrees Celsius), and D is larval density (number per square metre). Variables were retained in the model if their regression coefficients had t values that were significant at $P < 0.15$:

Variable	Regression coefficient	SE	Student's t
Intercept	175.5	±57.3	3.06[a]
L_2	2.4	±1.4	1.67[b]
$L_2 \cdot D$	0.7	±0.5	1.65[b]
$L_2 \cdot p$	0.3	±0.1	2.68[a]
$D \cdot p$	21.3	±8.2	2.57[a]
$T \cdot D$	5.2	±1.7	3.10[a]
$L_2 \cdot T$	0.8×10^{-1}	±0.2	3.71[a]

[a]Significant at $P < 0.05$.
[b]Significant at $P < 0.15$.

The regression's F value is 3.66 ($P < 0.05$) and the coefficient of multiple determination (R^2) is 0.69. Neither skewness nor kurtosis is significant at $P > 0.05$.

The equation predicts that among populations of southern brook lamprey from streams characterized by an annual mean temperature of 15°C and pH 7.2, the percentage of males can be expected to decline as larval density increases provided individual growth rates are slow (Fig. 2). When growth is rapid, the percentage of males changes very little with larval density. The percentage of males is, at each larval density, less when growth is rapid than when it is slow. At pH 6.0 and 15°C, the equation predicts that among populations in which individuals are slow growing, the percentage of males will decline slightly as larval density increases (Fig. 2). In marked contrast, the percentage of males increases with larval density when individual growth is rapid. At pH 6.0, as is the case at 7.2, the percentage of males, at a given larval density, varies inversely with larval growth. It is noteworthy at pH 6.0 that the equation predicts the absence of males from populations in which growth is very high but larval density is low.

Southern brook lamprey are found typically in streams in which the annual mean temperature ranges from about 15 to 20°C. Over this range the regression predicts relatively little effect of temperature on population sex ratio when growth is slow and pH is low. However, in populations that exhibit rapid growth, the percentage of males varies inversely with temperature. Thus, among populations in which the modal length of larvae at 24 mo is 65 mm from streams of pH 6.5 with annual mean temperatures in the range of 15–20°C, the percentage of males is approximately 53% (Fig. 3). Under conditions that promote more rapid growth, the percentage of males decreases linearly, with the rate of decline being greater at 20 than at 15°C. Thus, in populations in which the modal length of larvae at 24 mo is 100 mm, the percentage of males is reduced to about 20 and 6 at 15 and 20°C, respectively.

Discussion

Variations in sex ratios of adult lamprey have been reported for both parasitic (Wigley 1959; Hardisty 1961; Beamish and Potter 1975; Stier and Kynard 1986) and nonparasitic species (Dean and Sumner 1898; Hardisty 1954, 1961; Purvis 1970). Early in his investigations, Hardisty (1954) suggested that the

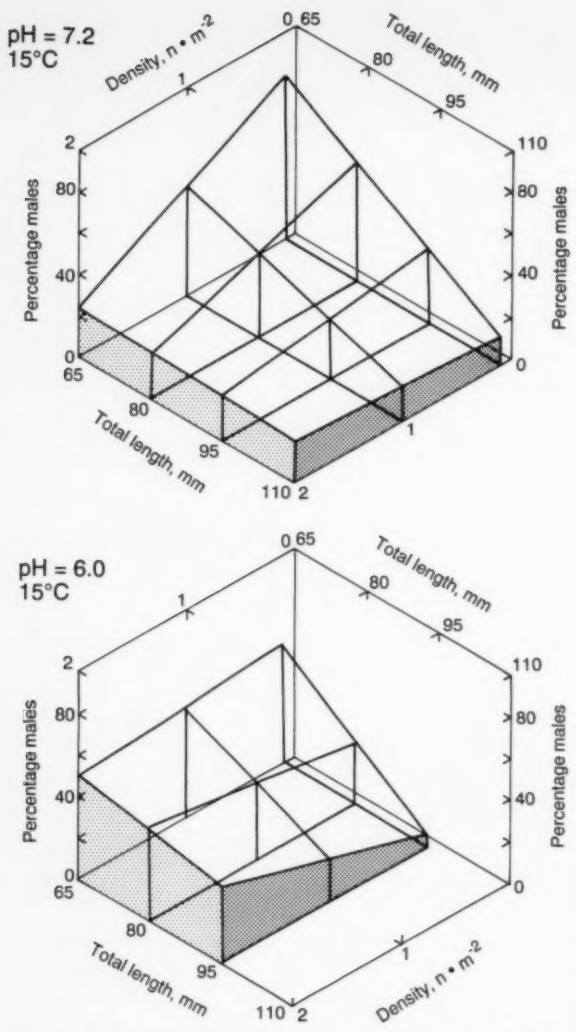

FIG. 2. Predicted population sex ratio, as percentage of males, in relation to larval density and growth, the latter as total length at 24 mo of age.

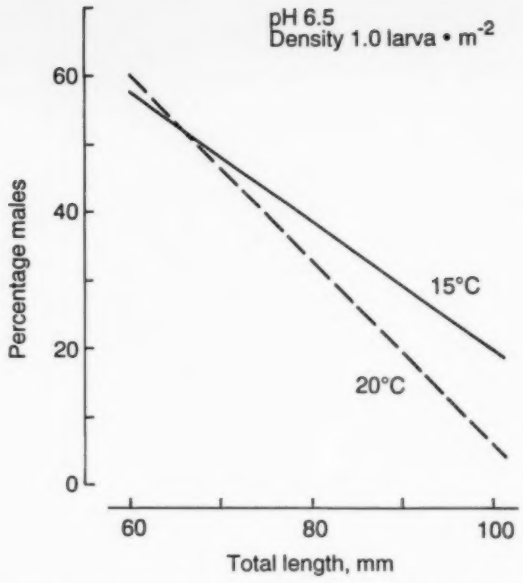

FIG. 3. Influence of annual mean stream temperature and larval growth (length at 24 mo) on population sex ratio when density of larvae is $1 \cdot m^{-2}$ and ambient pH is 6.5.

variation in sex ratio of adult brook lamprey, *Lampetra planeri*, might be due to the effect of the environment on the course of gonadal differentiation. In a subsequent study, Hardisty (1960) examined small collections of *Lampetra fluviatilis* larvae from two streams, a small sample of sea lamprey larvae from one stream, and a large sample of *L. planeri* larvae from a single stream and found sex ratios to vary only between 42 and 49% males. He concluded that these differences from parity were not sufficient to support his earlier view of environmental sex determination. The direct association between percentage of adult male lamprey and their respective population size was attributed to differences in adult recruitment or sex-related mortality. Hardisty's (1960) conclusion was, in part, based on the assumption that most mortality after the prolarva interval occurs during metamorphosis or during the period of sexual maturity. Research currently underway on mortality of southern brook lamprey indicates quite significant rates of instantaneous natural mortality during larval development in some streams.

Long-term studies of the landlocked sea lamprey in the upper Great Lakes have found sex ratios to vary widely with relative abundance. When abundance was high following their invasion of the upper Great Lakes, adult males typically composed 60%, and in some years as much as 70%, of the population (Heinrich et al. 1980). As lamprey numbers were dramatically reduced following the application of TFM, the proportion of adult and larval males correspondingly declined and a predominance of females emerged (Smith 1971; Purvis 1979; Heinrich et al. 1980). No evidence is available to suggest that TFM is differentially toxic to the two sexes (Purvis 1979; NRC 1985). Sea lamprey populations naturally characterized by low densities similarly show an excess of females (Purvis 1979). Recently, M.F. Docker and F.W.H. Beamish (personal observation) found that sex ratios of larval least brook lamprey varied directly with relative abundance from 24 to 71% males. These patterns of increase in the proportion of larval and adult male lamprey with population size are similar to that found for larval southern brook lamprey except among populations where individual growth was slow in which case the pattern was reversed.

Somatic growth of southern brook lamprey larvae is, for most populations, similar throughout the year. Extensive collections of larvae from Choclafaula Creek demonstrated little seasonal variation in growth reflecting the combined effects of only moderate temperature changes and fluctuating stream discharge (Beamish 1982). Accordingly, statolith growth in larvae from Choclafaula Creek was uniform and annuli did not form (Beamish and Medland 1988). Failure to recognize statolith banding patterns in other populations presumably also indicates even growth throughout the year. Only in Binion Creek was seasonal growth sufficiently variable to provide recognizable annuli in statoliths.

Support for environmental sex determination is strengthened by observations in this study and in that by Docker and Beamish of similar sex ratios among age groups within streams. This

observation refutes earlier suggestions of differential mortality between the sexes accounting for skewed population sex ratios. A more appealing mechanism is suggested by the observations made by Epple et al. (1982). They found that circulating steroids in larval sea lamprey responded to crowding. Of particular interest to the argument of environmental sex determination was the direct association between larval density and testosterone concentration. It is worthy of note that Docker (1992) was unable to direct the course of gonadal differentiation by exposing lamprey to any of several gonadal steroids including testosterone. Thus, while the course of sex differentiation may not be susceptible to manipulation by exogenous hormones, the influence of endogenous hormones remains largely unknown (Docker 1992).

The percentage of female southern brook lamprey varied directly with growth under most conditions, the effect being most pronounced when larval density was low and annual mean temperature high. Docker and Beamish did not find a significant relationship between the percentage of females and age group modal length. They did, however, find that fecundity of least brook lamprey increased with the cubic power of total length and also that fecundity, adjusted to a specific length, varied directly with the individual growth rate that characterized each population. It is interesting that the efficiency of fertilization or egg deposition in lamprey is enhanced by the presence of more than a single male (Manion 1968; Hardisty and Potter 1971). Precisely how population size is regulated in populations with a predominance of females is beyond the scope of the present study, but fertilization efficiency may represent one important mechanism.

Male southern brook lamprey were more prevalent under acidic than alkaline conditions, except under conditions of low larval density. A similar effect of pH has been reported for some teleost fishes. A particularly dramatic effect was reported by Heiligenberg (1965) who found the offspring of *Pelvicachromis* to be all male in acidic and all female in alkaline water. Similarly, Rubin (1985) found the offspring from five cichlid species and green swordtail, *Xiphophorus helleri*, to be almost entirely male at low pH and mostly female at neutral pH. The mechanism by which this occurs has not been described, but low pH can influence reproduction in fishes by decreasing the motility of sperm, through damage to genetic material in the developing ova, and by inhibition of hormone production or activity (Fritz 1980; Haines 1981).

Temperature has been implicated in sex differentiation in many lower vertebrates including fish (Conover and Kynard 1981; Conover 1984; Sullivan and Schultz 1986) and reptiles (Yntema 1979; Bull 1980; Deeming and Ferguson 1988). These studies and others suggest that temperature acts early in embryonic or larval development to influence the outcome of primary sex differentiation rather than causing a secondary reversal of gonadal sex. There is some evidence that temperature-dependent sex determination in at least the Atlantic silverside, *Menidia menidia*, is adaptive in that the environment that offspring enter has a gender-dependent effect on fitness (Charnov and Bull 1977; Conover 1984). Sullivan and Schultz (1986) found that in the teleost *Poeciliopsis lucida*, some females are influenced by ambient temperature to produce skewed sex ratios among the offspring, while other females produce approximately equal numbers of male and female progeny. Apparently in *P. lucida*, population sex ratio is influenced by both genetics and environmental quality. In the present study, temperature was not measured during egg incubation or during early larval development. Only the annual mean temperature was estimated which may or may not reflect the thermal regime during early development. It is important to point out that in lamprey, sex is not differentiated for about a year, in contrast with that found for many animals including the Atlantic silverside which completes its life cycle within a single year.

Polygenic sex determination is thought to be the primitive genetic mechanism for sex determination and to have been replaced during the course of evolution first by genic sex determination and then by semichromosomal or chromosomal sex determination (Kirpichnikov 1981; Rice 1986). In the polygenic system the sex of an individual reflects the cumulative effect of environmental quality acting on each of a large number of genes which individually exercise only a small influence on sex. Polygenic sex determination is thought to be rare in extant species (Rice 1986), and sex chromosomes are either absent or in a primitive state of differentiation in fish (Ohno 1967). It is interesting that in the hermaphroditic fish *Rivulus marmoratus*, which lacks sex chromosomes, the gonad develops into a testes rather than ovotestes when ambient temperature is low (Harrington 1971). This suggests that in at least some lower vertebrates, gonadal differentiation is affected by metabolic rate and, by implication, growth.

Environmental sex determination has been proposed as a tactic to optimize fitness in relation to environmental quality (Charnov and Bull 1977). Thus, if sex ratios are adaptive in gonochoristic vertebrates, individuals of large size would be expected to become the sex in which the rewards for being large are greatest or the penalty for being small least (Charnov and Bull 1977; Conover 1984). In populations of both the southern and least brook lamprey, there was seldom a significant difference in length between males and females of the same age. Docker and Beamish (personal observation) suggested that the mechanics of spawning may result in reduced fertilization efficiency when there are large differences in size between individuals. Malmqvist (1983) found that fertilization in *L. planeri* was most successful when length differences between the two sexes were less than 14%.

If sex ratios are indeed adaptive, they may be expected to vary throughout stream length in response to changes in environmental quality. Southern brook lamprey are typically found in relatively small streams in which environmental quality is reasonably homogeneous throughout their entire length. In this context, the present study did not compare sex ratios at specific locations within a stream. However, sex ratio of least brook lamprey was examined at several locations within each of three streams by Docker and Beamish and significant differences were not found.

In summary, the present study provides further evidence of environmental sex determination in the phylogenetically primitive lamprey and attributes the response to several abiotic and biotic factors. It remains unclear whether sex ratios in lamprey are or are not adaptive. Sullivan and Schultz (1986) offered the possibility that sex ratios may simply reflect the outcome of a series of physiological and endocrinological processes without a specific linkage to fitness.

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Structure of the Body-Size Spectrum of the Biomass in Aquatic Ecosystems: A Consequence of Allometry in Predator–Prey Interactions

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An equation describing predator–prey trophic energy transfers and production within the body-size spectrum of the biomass of aquatic ecosystems is formulated using allometric functions of body size. Its solution is the sum of two parts. One is a quadratic term that gives parabolic domes of biomass, in accordance with observations in nature. A second part, which seems not to have been recognized earlier, is a periodic function of log body size having significant potential for interpreting sample data reflecting ecosystem dynamics. The formulation is fitted to fish data from a small lake to demonstrate the applicability of the basic model to observations and to examine the scales of interaction of the measures of ecosystem dynamics that may be derived from them.

Nous formulons une équation décrivant les transferts d'énergie trophique entre prédateurs et proies et la production dans le spectre de dimensions corporelles de la biomasse des écosystèmes aquatiques à partir des fonctions allométriques des dimensions corporelles. La solution de cette équation est la somme de deux termes. Le premier terme, quadratique, explique la variation parabolique de la biomasse, conformément aux observations faites dans la nature. Le second terme, qui ne semble pas avoir été reconnu antérieurement, est une fonction périodique du logarithme de la dimension corporelle qui offre un potentiel important pour interpréter les données d'échantillonnage reflétant la dynamique d'un écosystème. La formulation est ajustée aux données recueillies sur les poissons d'un petit lac pour démontrer l'applicabilité des modèles de base aux observations et pour examiner les échelles d'interaction des mesures de la dynamique écosystémique que l'on peut en tirer.

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The structure of the body-size spectrum of biomass in aquatic ecosystems reflects general features of the underlying dynamics (Boudreau et al. 1991). However, while this spectrum has obvious parallels in the corresponding production spectrum that provides a measure of energy flux (Dickie et al. 1987), the mechanisms relating them, and their consequent sensitivity to population and environmental effects, need to be made clear. Until the mechanisms are understood, we cannot rely on the observable biomass spectrum for information on the more elusive mass and energy fluxes.

In this paper, we develop a simple equation to describe the transfer of mass between prey and predator elements in an ecosystem. We infer from various sources that the identified fluxes are body-size dependent. That is, they are related to the characteristic body-size-dependent metabolic rates and densities of organisms (Boudreau et al. 1991). Utilizing these underlying dynamics, major features of the biomass spectrum are then shown to follow as mathematical consequences of the production relations. The results support using the biomass spectrum as a tool in comparative ecology.

Predator–Prey Equation

In aquatic ecosystems, there is a net movement of matter and energy upward through the biomass spectrum from small to larger producers through a succession of trophic linkages. The predator–prey equation we develop describes the linkage between two feeding groups labeled i and j for prey and predator, respectively.

Members of a feeding group are considered to have similar feeding habits and diets and a limited, but not necessarily small, range of body sizes.

Let $\beta_i(w_i)$ = total biomass of organisms of sizes $\leq w_i$ in feeding group i and $\pi_i(w_i)$ = total production of organisms of sizes $\leq w_i$ in feeding group i. Similar definitions hold for a feeding group j. Biomass and production are averages observed over a period of time in a spatial region representative of the ecosystem and are measured on a per unit area basis. Energy and mass are related by a mass-caloric conversion factor and hence are regarded as equivalent.

Treating body size as a continuous variable, we have for the biomass and production due to organisms in the size interval Δw_i about w_i the estimates $(d\beta_i(w_i)/dw_i)\,\Delta w_i$ and $(d\pi_i(w_i)/dw_i)\,\Delta w_i$, respectively.

For illustration, we may consider two groups, i and j, making up an exclusive one-to-one predator–prey relationship. The simplest model then posits that the predators in a given size interval Δw_j centered about w_j graze on the prey in a propor-

tionally sized interval Δw_i centered about w_i. The size intervals are neither necessarily contiguous nor small.

We define the "feeding size ratio" w_i/w_j, considered as a function of w_j. It is an average of the ratio of body sizes between the two groups and is a characteristic of the predator feeding group. We emphasize that the average is over a possibly broad distribution of feeding size preferences. This important feature of predator–prey interactions is considered below in relation to the concept of "trophic position." In aquatic systems the average feeding size ratio function $R_j(w_j) = w_i/w_j$ generally has an upper bound much less than 1 (Sheldon et al. 1972; Kerr 1974). Similarly, the "size interval ratio" $\Delta w_i/\Delta w_j$ is considered a characteristic function of the predator feeding group. However, the only parameter of the distribution of feeding size preferences required in the development of the model is the average feeding size ratio function.

The model estimates production within the interval Δw_j of the predator group to be directly proportional to the biomass of the prey group within Δw_i. This means, for example, that if by chance the prey biomass density in an area were abruptly increased, there would be a correspondingly abrupt increase in predator population production, followed by a slower responsive increase in the predator biomass density until it comes into balance with the food supply rate at the former level of individual ration. In fact, as pointed out by Dickie et al. (1987), we infer from the regularity in specific production (see definition below) with body size in natural populations that the organisms within any feeding assemblage adjust their local density so that the individual's ration is in balance with its "routine" metabolic requirements (Winberg 1956). Accordingly, we write the predator–prey equation

$$\frac{d\pi_j(w_j)}{dw_j}\Delta w_j = K_j(w_j) f_{ji}(w_j) \frac{d\beta_i(w_i)}{dw_i}\Delta w_i$$

where the proportionality constant is separated into two parts, a gross production efficiency $K_j(w_j)$ and a predator-induced mortality rate $f_{ji}(w_j)$. $K_j(w_j)$ is a dimensionless quantity ≤ 1 representing the gross proportion of catch (biomass) from the prey that is converted to predator biomass. $f_{ji}(w_j)$, with the dimensions of (time^{-1}), is the specific mortality rate of the prey group due to grazing by the predator group (Dickie et al. 1987).

"Specific production" in the predator group is defined as the ratio of incremental production to incremental biomass:

$$P_j(w_j) = \frac{\dfrac{d\pi_j(w_j)}{dw_j}\Delta w_j}{\dfrac{d\beta_j(w_j)}{dw_j}\Delta w_j} .$$

Specific production is the reciprocal of the "turnover time" of the predator.

It is convenient to introduce a notation for the differential form, or "biomass spectral density," of the biomass functions:

$$B_n(w) = \frac{d\beta_n(w)}{dw} , \qquad n = i, j .$$

This definition is equivalent to the specification of biomass per unit body size. It is useful in theoretical modeling, and biomass distributions using it are sometimes referred to in the literature as "normalized biomass spectra."

Because the model assumes that the prey-predator size interval ratio is a characteristic function of the predator, the ratio can be absorbed into f_{ji} and the i subscripts dropped. Thus, we define the predatory "grazing coefficient"

$$F_j(w_j) = f_{ji}(w_j)\frac{\Delta w_i}{\Delta w_j}$$

as the grazed fraction of the biomass of prey of size w_i, corresponding to that specified by the average feeding size ratio, R, multiplied by a prey-to-predator concentration factor $\Delta w_i/\Delta w_j$ which allows for the fact that there may be differences in the proportional size range of the predator and prey feeding groups. The basic equation we wish to consider, describing specific production in the predator group in relation to the biomass of prey and predator organisms, then becomes

$$(1) \quad P_j(w_j) = K_j(w_j)F_j(w_j)\frac{B_i(w_i)}{B_j(w_j)} .$$

Allometry of the Production Parameters

Enquiry into the dynamics of the biomass spectrum is simplified by recognizing allometric relationships between body size and the terms included or implied in eq. (1). An allometric relationship between two quantities translates into a straight-line relationship in a log–log plot. When the independent variable is the log of body size, the "slope" of the allometric relationship is identical to the slope of the line. In the case of "specific production," for example, its relationship to body weight is well recognized as reflecting a basic allometric relationship between metabolic rate and body size (Banse and Mosher 1980; Peters 1983). For our predator–prey model of feeding groups related according to eq. (1) by the predator production function, we propose to treat all production-related terms as allometric functions of body size.

We define the relationship between specific production and body size as

$$(2) \quad P_j(w_j) = a' w_j^{\gamma'}$$

where γ' is the (negative) slope of specific production, a characteristic parameter of the ecosystem.

In development of the equations in this paper, however, we need to recognize the existence of two allometric relationships of specific production to body size, depending on the scale of aggregation of the measurements of the individual organisms. As is illustrated in Fig. 1, all the organisms in an aquatic ecosystem can be classified into quasi-taxonomic groups, often referred to as phytoplankton, zooplankton, small invertebrates, fish, etc., which fall within characteristic ranges of body size. Following the discovery of Humphreys (1979) that the organisms within these defined groups may have a variety of feeding habits, but in their natural environments display a uniform production efficiency (Dickie et al. 1987), we term these size groupings of the organisms "trophic positions" (Levine 1980). A trophic position thus comprises a group of organisms of various species, falling within a given range of body sizes, and normally playing a characteristic role in the energy flow process.

Within a trophic position, all zooplankton for example, we may distinguish different feeding groups with different feeding habits and diets, hence occurring at different trophic levels within linearly arranged food chains. In certain cases the range

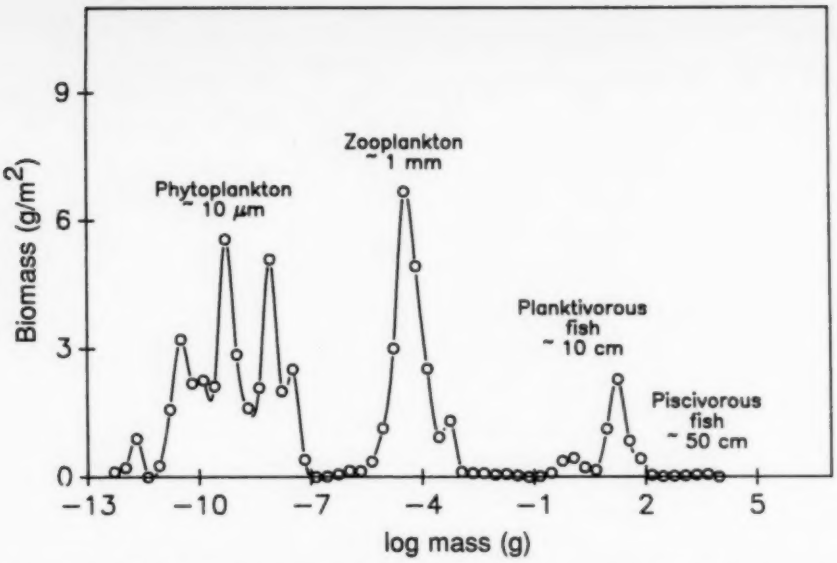

FIG. 1. Example of a body-size spectrum of the biomass, copied from Sprules et al. (1991) and compiled from their sampling of Lake Michigan, showing the component trophic groups.

of sizes within successive positions may be so broad that some prey are larger than some predators, and cases in which predators feed on prey larger than themselves are not uncommon. We consider such situations in more detail in Thiebaux and Dickie (1993), as multispectral models, and return to their consideration below in the section entitled Feeding between trophic positions: multiple feeding groups. Meanwhile, we emphasize that the classification of a group of organisms into trophic positions is based on the observation of their common production efficiency in the ecosystem.

In fitting data on specific production for organisms in natural communities, Dickie et al. (1987) found that if trophic position is not distinguished, so that the organisms are treated statistically as a collective whole, the allometric relationship of specific production with body size has a low negative slope. This slope has been called a primary slope because the statistical treatment of organisms as individuals of different sizes reflects the influence on the energy flux of their individual physiologies, expressed in their metabolic rates (Boudreau et al. 1991).

On the other hand, if the individuals are separated and treated according to their trophic positions, allometric relationships between specific production and body size within the trophic positions are found to have more steeply negative slopes, traced to their classification into groups having common production efficiencies. These secondary slopes are approximately equal for all trophic positions but the intercepts are different. The statistical treatment now reflects ecological effects of changes in density with body size, added to the metabolic effects. Under this classification, it thus appears that the data reflect two hierarchical levels of the underlying dynamics (O'Neill et al. 1986).

In the following development, we initially consider feeding linkages between a predator and its prey as they would be described by a single biomass spectral density function, hence within a single trophic position where it is the secondary or ecological slopes that are of greatest relevance. These results are then generalized to the case of linkages between trophic positions along the primary slope (Thiebaux and Dickie 1993).

As stated above, in addition to the basic allometric form for specific production, we apply allometric representations to the remaining production-related components in eq. (1): the gross production efficiency $K_j(w_j)$, the predatory grazing coefficient $F_j(w_j)$, and the average feeding size ratio function $R_j(w_j)$ (Dickie et al. 1987). In each case, we need to identify the two levels of scaling of the ecosystem dynamics and recognize that there are constraints on the applicability of allometric relationships to some of them.

The gross production efficiency function, K_j, has been subjected to many experimental studies since the original formulations of Ivlev (1960). At the primary or overall scale, individual growth efficiency of a predator has been widely recognized as dependent on the relative mass of its ration but independent of predator body size (Lavigne 1982; Platt 1985). Paloheimo and Dickie (1965) defined feeding habits within "stanzas," often representing the selection of particular food species. They found the growth efficiency curve, or K-line, to have a steep negative slope when the food was of relatively small particle sizes, such as when fish in one trophic position feed on invertebrate prey in another trophic position.

Translation of effects at this individual scale into the ecologically significant population production efficiency is more complex. For example, for a predator at a given trophic position, with a single diet and a single level of availability, the K-line may be equally predicted from predator ration or body size. When it is treated as an allometric function of body size, the expected (negative) slope is, in absolute value, equal to or greater than the allometric slope of metabolism with body size (Dickie et al. 1987).

As a fish predator grows, it characteristically shifts from feeding on the abundant, small invertebrate prey to more catchable, larger vertebrate prey. This shift from feeding across trophic positions to feeding on the smaller fish within the same trophic position gives rise to an upward shift in the position and a lowering of the slope of the K-line. Kerr and Martin (1970) found that such efficiency shifts may be large enough to give

rise to increases in average production efficiency, a fact of significance in connection with assessing the validity of fitted biomass spectrum models. That is, as fish predators shift from feeding on small to large food particle sizes, they may more than compensate for decreases in the rate of encounter by increases in the energetic efficiency of the food intake. This indicates that there are interactions between the growth efficiency and the grazing mortality terms in the production equation.

The grazing mortality coefficient, F_j, itself, is difficult to measure and is not well known for natural populations. Dickie et al. (1987) concluded that it must have an overall or primary allometric slope of the order of -0.25 between the average body sizes of successive trophic positions, i.e., for an average feeding size ratio, R, of the order of 10^{-4} or 10^{-5}. That is, the primary allometric slope of the combined conversion coefficient, K_jF_j, must have a negative value of the same order of magnitude as the primary slope of the specific production.

The situation for K_jF_j, with respect to feeding linkages at the secondary level, within trophic positions, is less clear. As noted above, there are known situations where prey may be larger than their predators. The numerical value of the secondary allometric slope of F_j is problematic because it will depend on the size-specific rate of growth and rate of change in density of the prey, as well as on the breadth of body size of the prey species complex utilized by the predator. However, we do know that population food consumption decreases with predator body size at about the same rate as the metabolic rate (Dickie et al. 1987). Hence, with increases in food particle size the grazing mortality term must also decrease rapidly. Where the K-line is relatively flat, a constant production efficiency indicates that even steep drops in F_j are compensated by availability changes. Given that a constant production efficiency characterizes a trophic position, it appears that the combined secondary grazing mortality and production efficiency functions are likely to have a negative allometric slope equal to or greater than the slope of the corresponding specific production.

In what follows, the allometric relationships for production efficiency and the population grazing coefficient are combined into a single proportionality term of the form

$$(3)\quad K_j(w_j)F_j(w_j) = a''w_j^{\gamma''}$$

and treated on the appropriate scale.

With respect to the average feeding size ratio function $R_j(w_j)$, the data suggest that within stanzas of the food selection sequences of predators, predator and prey body sizes tend to increase together within a growing season. That is, consistency in R_j will depend on the relative rates of growth of predator and prey. Between stanzas, which may often be coincident with changes in seasons or distribution patterns, there may be more abrupt changes in the size ratio. Given such observations, we treat R_j as an allometric function of body size according to the formulation

$$(4)\quad R_j(w_j) = \frac{w_i}{w_j} = Rw_j^{\rho}\ .$$

In what follows, we initially consider the stationary situation where R_j is a constant by letting $\rho = 0$. Later, we consider effects of modifying it, utilizing different values of R or ρ in particular feeding situations, such as may frequently occur where there are restricted size ranges of the food organisms available during the annual feeding and growing seasons.

It should be noted that our use of a constant average feeding size ratio is based on the mathematical idea that such predator attributes constitute an elementary type of "similarity," i.e., where predators feed on prey having a range of sizes described by a fixed distribution of prey/predator size ratios. As a hypothetical example, if organisms across the size spectrum in an ecosystem graze on all organisms less than 1% of their own body size, then we are dealing with a type of predation similarity.

Some caution is required in applying the allometric rule to quantities with specified or intrinsic upper bounds. Gross production efficiency and the average feeding size ratio function are both bounded by unity and very likely by some number less than unity. If the allometric law relating these functions to body size w yields anything other than constant values, then there must be a correspondingly restricted domain of applicability in w. Otherwise, these functions would increase without limit as either $w \to 0$ or $w \to \infty$. Given these qualifications, eq. (1), (2), (3), and (4) make up a system describing mass transfer within or between trophic positions of an ecosystem.

Our approach to understanding the dynamics of an ecosystem is to assume that predation similarity, as characterized by an average feeding size ratio, and the consequent allometric relationships, all of which are directly related to production, are the fundamental elements that drive the system through the predator–prey interaction equation with a consequent distribution of biomass.

Feeding Linkages within a Trophic Position

A model of a feeding linkage within a single trophic position applies, for example, to the common situation where large fish feed on small ones (Kerr 1974; Jones 1984). Using a constant average feeding size ratio, we can write $w_i = Rw_j$, drop the subscripts used to distinguish feeding groups, and rewrite eq. (1) as

$$\frac{B(w)}{B(Rw)} = \frac{K(w)F(w)}{P(w)}\ .$$

In logarithmic form, and using the allometric eq. (2) and (3), we get

$$(5)\quad \log B(w) - \log B(Rw) = \log a + \gamma \log w$$

where

$$(6)\quad a = \frac{a''}{a'} \quad \text{and} \quad \gamma = \gamma'' - \gamma'\ .$$

The solution of eq. (5) for the spectral density function $B(w)$ becomes transparent if we let $y(x) = \log B(w)$, with $x = \log w$:

$$y(x) - y(x + \log R) = \log a + \gamma x\ .$$

This is a functional equation whose solution is the sum of a particular quadratic in x plus a function that is periodic in x. Reverting to the B and w notation, we can write

$$(7)\quad \log B(w) = \log B_0 + \frac{1}{2}c_0\left[\log\frac{w}{w_0}\right]^2 + H(\log w)$$

where B_0 is a free constant and the second term is a quadratic function of the logarithm of the predator body size w. Together the two terms form a parabola that we can interpret as a smooth background component of the biomass spectral density. The last

term $H(\log w)$, on the right-hand side, is any periodic function with period = $|\log R|$. It arises from the nonlocal nature of the predator–prey interaction across the gap $|\log R|$ and is treated in some detail by Thiebaux and Dickie (1993). Its significance is further discussed below.

With respect to the quadratic part, by comparing with eq. (5), we find

$$(8)\quad c_0 = \frac{-\gamma}{\log R}, \quad w_0 = \frac{\sqrt{R}}{a^{1/\gamma}}.$$

The second derivative, $d^2y/dx^2 = c_0$, is the curvature at the vertex of the parabola and must be negative in order that the system contain a bounded biomass spectral density and a finite biomass. As it is safe to assume that $\log R$ is negative, it follows that γ must also be negative. Hence, γ'', the slope of the combined production and grazing efficiencies, must be even more negative than γ'.

Ignoring the periodic part, we conclude that the body-size spectrum of the biomass of predator–prey assemblages governed by eq. (5) will form a background parabolic dome as a mathematical consequence of the feeding relationships established within the trophic position. The vertex or peak of this dome occurs at $(\log B_0, \log w_0)$. It is worth noting that B considered as a function of $x = \log w$ has the form of the normal curve

$$B \propto \exp\left[-\frac{|c_0|}{2}(x - x_0)^2\right].$$

The mass of a parabolic dome of vertex curvature c is readily computed:

$$\text{Biomass of dome} = B_0 D$$

where

$$D = \sqrt{2\pi \ln b} \cdot \frac{w_0}{\sqrt{|c|}\, b^{1/c}}$$

and b is the logarithm base. D is a measure of the width of the dome in mass units.

With the exception of its periodicity, the periodic term H in eq. (7) is indeterminate within the model framework. However, its existence has important practical implications for interpreting observations of biomass distributions. Its average value (if an average exists) could be absorbed into B_0 leaving a residual that oscillates with period $|\log R|$ about the basic quadratic part of $\log B$.

These oscillations in the biomass within trophic positions would be stable in a simple model where the average feeding size ratio has no variance. On the other hand, with some variance due to a range of selected foods, a negative feedback mechanism would likely dampen the oscillations. For example, if an organism on the crest of an oscillation feeds on particles *near* rather than precisely *on* a neighbouring crest, it would have smaller densities of prey than the simple model requires to maintain this departure from the quadratic part. The continued existence of such oscillations would depend on interactive features of predator foraging behaviour and prey availability, which might be verifiable by detailed study. In general, however, variance in R about the various average values would produce a smoother spectrum than the simpler model predicts.

The fact that the model predicts a periodicity or oscillations about the biomass curve but can really say nothing more about them should not be construed as indicating negligible importance. The oscillations may even appear as dominant features in an observed ecosystem, as is implied by Thiebaux and Dickie (1993). More generally, we might expect them to appear as perturbations superimposed on the curve of biomass, hence with a period shorter than the width of the dominant biomass parabola related to the quadratic term. They may arise from a number of phenomena intrinsic to the biological system, or to events extrinsic to the system, such as may result from the action of time-periodic environmental phenomena on the body-size distribution.

It should be recognized here that this model is stationary in time; hence, the oscillations under discussion are not in time; they are functions of body size alone. We recognize that real systems are affected by time-dependent factors which will be reflected in the sampling data used to fit the equations and wish to point out that because of the twofold scaling of the production processes, there are two different criteria of the state of equilibrium that affect interpretation of the fittings. In the overall spectrum case, characterized by the primary slope, it may be necessary to approximate a long-term "population" equilibrium by averaging over variations in cohort abundance on the time scales described by Denman et al. (1989). This has been taken into account by Boudreau and Dickie (1992).

In the case of the biomass spectrum characterizing a single trophic position, the time scales of energy transfer are determined on much shorter scales. Predator–prey equilibrium *densities* would appear to be determined on the scale of days or weeks (Hochachka and Somero 1971), required for predators to acclimate their activities to the level at which individuals can satisfy their requirements from the existing levels of food availability.

Feeding between Trophic Positions: Multiple Feeding Groups

Given biomass domes arising within trophic positions, we need to examine the important energy fluxes between positions. In practice, significant prey and predator feeding groups labeled i and j, respectively, often reside in different trophic positions, describable by biomass spectral density functions that would be independent functions were there no feeding linkages between them. In considering this aspect of feeding linkages, we continue to assume predation similarity, as characterized by a constant average feeding size ratio, and allometric forms for overall production processes that would apply between trophic positions. Eq. (1) becomes

$$(9)\quad \log B_j(w) - \log B_i(Rw) = \log a + \gamma \log w.$$

This equation has multiple solutions, labeled $i, j, \ldots$, in contrast with eq. (5) with its single-valued solution. It is evident that if the biomass spectral density of one feeding group is arbitrarily specified, then the spectral density of the other feeding group is predicted by eq. (9). The contrast between equations such as (5) and (9) is discussed at length in Thiebaux and Dickie (1993).

Suppose we take the predator spectral density to be a parabolic dome with a vertex of curvature c located at $(\log w_{j0}, \log B_{j0})$:

$$(10)\quad \log B_j(w) = \log B_{j0} + \frac{1}{2} c \left[\log \frac{w}{w_{j0}}\right]^2.$$

Then, eq. (9) predicts that the corresponding prey spectral density is the parabolic dome

$$\log B_j(w) = \log B_{j0} + \frac{1}{2} c \left[\log \frac{w}{w_{j0}} \right]^2 .$$

That is, the prey spectral density has the same curvature as the predator dome, but its vertex is located horizontally at

$$\log w_{j0} = \log w_{j0} - h$$

where

$$(11) \quad h = -\frac{\gamma}{c} - \log R$$

and vertically at

$$\log B_{j0} = \log B_{j0} - \left[\frac{\gamma^2}{2c} + \gamma \log w_{j0} + \log a \right] .$$

It is noteworthy that the vertical steps between peaks of successive domes are evidently independent of the average prey/predator body size ratio R. We shall assume that the peaks are "normally" spaced in the sense that $h \geq 0$. This means that the peak of the prey dome lies to the left of the peak of the predator dome.

If the biomass spectrum of the initial feeding group containing the predator is a parabolic dome of the type described in the previous section, then the biomass spectrum of the predicted prey group is also a parabolic dome, having the same curvature (i.e., shape) but displaced horizontally and vertically from the initial dome. Parabolic domes of approximately the same shape are often observed in nature (Boudreau et al. 1991). Indeed, mathematically speaking, parabolas are the only shapes which would be replicated *without distortion*, according to the similarity rule embodied in eq. (9), across linked feeding groups in the ecosystems for which this equation applies.

The most important additional feature of the multiple spectral case lies in the fact that the separate feeding groups can now have overlapping body-size domains, in which some predators will be smaller than some prey. This may be a critical feature in the interpretation of the sampling data for observed spectra. As noted by Thiebaux and Dickie (1993), empirical determination of the multispectrum case may not be a simple matter, but in the majority of situations there would appear to be little error involved in fitting them as a single function.

Variation in the Average Feeding Size Ratio

To assess the generality of the foregoing, it is necessary to examine the effects of relaxing the simplifying condition of a constant average feeding size ratio, R. In what follows, we continue to use the allometric form, eq. (4), to describe the relationship.

Let the predator and prey groups be labeled j and p, respectively. In place of eq. (9), we now have

$$\log B_j(w) - \log B_p (R w^{\rho+1}) = \log a + \gamma \log w .$$

Using eq. (10) to describe the given predator spectral density, we find once again that the predicted prey spectral density is a parabolic dome:

$$\log B_p(w) = \log B_{p0} + \frac{1}{2} c_p \left[\log \frac{w}{w_{p0}} \right]^2 .$$

The vertex curvature is

$$c_p = \frac{c}{(\rho+1)^2}$$

and the horizontal position is

$$w_{p0} = \log R + (\rho+1)\left(\log w_{j0} + \frac{\gamma}{c}\right)$$

but the vertical position is found to be independent of ρ and therefore the same as in the constant feeding size ratio case:

$$\log B_{p0} = \log B_{j0} - \left[\frac{\gamma^2}{2c} + \gamma \log w_{j0} + \log a \right] .$$

The persistence of parabolic domes in this more general case of the allometric feeding size ratio underscores the implication of this development that the parabolic dome form has a special status in ecosystem models, although the curvature of the domes may now be different among the feeding groups.

A special case arises if $\rho \to -1$. The curvature of the prey dome approaches $-\infty$, becoming a narrow line at the fixed body size $w_{p0} = R$. Thus the predators would be feeding on a group consisting of a single or a very narrow range in body size, possibly representing a particular highly preferred or especially available species.

Application to Lake Data

The biomass and production of all the fish in a small freshwater lake were measured by Chadwick (1976). Because all the fish were taken simultaneously, this data set is uniquely free from the problems of sampling bias that plague the interpretation of population data (Blackburn et al. 1990). We have selected it for this reason. If short-term physiological processes determine the structure of the biomass in secondary spectral domes, this data set should be representative of a fish ecosystem, within the limits imposed by the fact that it represents a single time slice of a relatively small environment. On the basis of available information, there is no reason to suppose that the data are not representative of small, temperate-zone Laurentian Shield lakes.

Figures 2 and 3 show the biomass density and specific production data calculated in unit log (decade) body-size intervals ranging from 10^{-3} to 10^{3} kcal equivalents. The parabolic dome shape of the observed biomass and the linear shape of the observed production are both evident in the figures. In this section, we fit our model equations to these field data, assuming that there will be feeding linkages of the fish, either as predator–prey relationships or competitors within a single trophic position, hence that they can be described within a single biomass spectral density function. In principle, the average feeding size ratio R and product Kf are determinable when the model is applied to such biomass and production data.

The goal of this analysis is to derive an independent set of model parameters by fitting to the lake data and then to check the reasonableness of the parameter values in light of any external evidence. While a single sampling does not provide a rigorous test of the usefulness of the biomass spectrum hypothesis, by this approach we can hope to understand some of the limitations to interpretation of features of a natural system that are imposed by our model simplifications.

The quadratic part of the modeled log biomass spectral den-

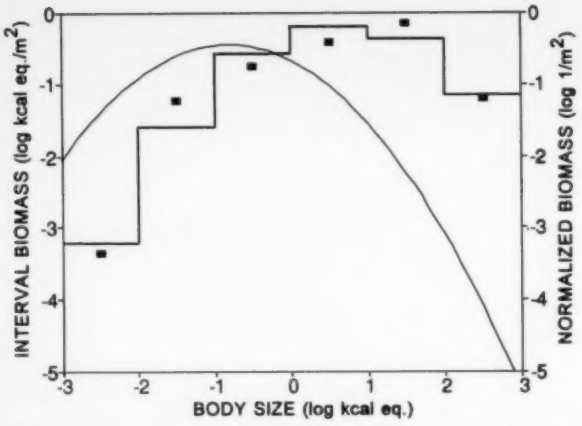

FIG. 2. Biomass density of fish in a small lake ecosystem. The squares represent observed biomass per square metre of lake surface in unit log body-size intervals. The smooth curve, scaled by the right-hand axis, is the quadratic part of the modeled (normalized) biomass spectral density. The stepped curve represents the integrated biomass within each body-size interval of the quadratic part of the biomass spectral density. The stepped curve was fit to the observed points. The relationship between the two curves is given by eq. (A1) where the smooth curve represents B_j, the stepped curve represents ΔM_j, and $k_j = 10$.

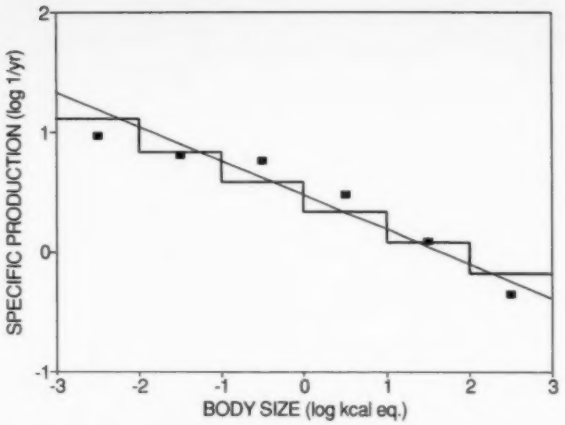

FIG. 3. Specific production of fish in the small lake ecosystem. The squares represent observed specific production in unit log body-size intervals. The straight line is the allometrically modeled specific production. The stepped curve represents specific production in each body-size interval computed from the modeled specific production and the modeled biomass spectral density shown in Fig. 1.

sity function, eq. (7), defined by the three parameters $\log B_0$, c_0, $\log w_0$, was determined by integrating trial quadratic functions over each of six body-size intervals determined from the data and selecting the best fit, in the least squares sense, of the log of integrated biomass values to the corresponding observed interval values. For given values of the parameters c_0 and $\log w_0$ the determination of the best $\log B_0$ follows from a standard linear regression. However, the determination of best values for c_0 and $\log w_0$ requires a nonlinear multiple regression method. In the current application, we chose an iterative search through trial values. The modeled solution for the quadratic part of the log biomass spectral density and its integrated interval values are shown in Fig. 2. (The relationshp between the two curves of Fig. 2 may be confirmed by reference to the development and notations for interval data in the Appendix, where the smooth curve represents $B_j(\hat{w}_j)$, the stepped curve represents $\Delta M_j(\hat{w}_j) = \hat{w}_j \Delta N_j(\hat{w}_j)$, and $k_j = 10$.)

The residuals between the modeled and observed interval values plotted in Fig. 2 could be interpreted as manifestations of the periodic part of the log biomass spectral density, having a (visually determined) period of approximately 3 to 4 log units. If this interpretation were correct the periodic component would yield the very reasonable average feeding size ratio $\log R \approx -3.5$, or possibly some other integral multiple of -3.5 units.

The period, amplitude, and phase of the lowest harmonic component of the periodic part could be estimated quantitatively by an extension of the nonlinear multiple regression. However, in this particular case, the limited sampling of biomass data does not seem to justify division of the data series into smaller size classes in an effort to support a more detailed analysis. For purposes of illustration, we are content to accept the above visual estimate while recognizing that a more rigorous test of the model is highly desirable and might be feasible given more extensive sampling of a homogeneous environment.

The observed specific production for each of the six body-size intervals is determined by dividing the observed production in each interval by the observed biomass in the same interval. Hence, observed interval production and biomass data are regarded as independent observations for the purpose of statistical error treatment. Observed interval production, used in fitting the modeled production as described next, is reconstructed by multiplying the reported interval specific production by observed interval biomass.

Similarly, modeled interval production is formed by integrating over each body-size interval the product of specific production, defined by the two parameters $\log a'$ and γ', and the modeled biomass spectral density found above. The best values of $\log a'$ and γ' are found by fitting in the least squares sense the log of the modeled interval production to the corresponding observed values. $\log a'$ is readily found by a linear regression, while γ' is found by an iterative search through trial values. Figure 3 shows observed and modeled specific production. The latter is displayed in two ways: specific production as a continuous allometric function, and interval specific production computed by dividing modeled interval production by modeled interval biomass.

The parameters a'' and γ'', defining the product Kf of the predator, are determinable from eq. (6) and (8) using the five parameters now available ($\log B_0$, c_0, $\log w_0$, $\log a'$, γ') together with assumed values of $\log R$. Figure 4A and 4B show the body-size dependence of Kf for $\log R = -3.0$ and -4.0, respectively. Corresponding parameter values are given in Table 1.

Standard errors for the model parameters in Table 1 were determined first by numerically computing the partial derivatives of the five parameters, evaluated at their best values, with respect to each of the 12 independent interval observations (6 biomass and 6 production observations), and second by assuming that the variances of the six observed interval biomasses were equal. With 3 degrees of freedom the estimate of this variance is $\text{var}(\log B) = 0.094$. Similarly, with 4 degrees of freedom the estimate of the (assumed equal) variances of the six observed interval productions is $\text{var}(\log P) = 0.074$. Finally, the standard errors of the parameters (or any functions of them such as a'', γ'', and Kf) are the square roots of their variances.

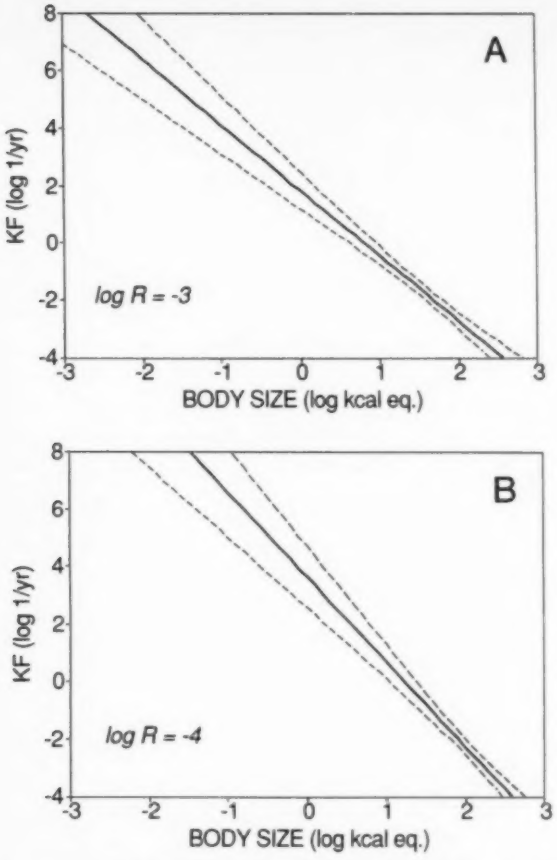

FIG. 4. Allometric body-size dependence of the product Kf of the predator's combined gross production efficiency and grazing coefficient found for the small lake ecosystem. Standard error limits are indicated by broken lines. Feeding size ratio log$R$ fixed at (A) -3 and (B) -4.

The latter are formed from linear combinations of var(log$B$) and var(log$P$) with coefficients that are products of the computed partial derivatives.

Because, as already noted, K is bounded by unity, the values of Kf obtained over roughly the upper half of the body-size range indicated in Fig. 4 appear reasonable. However, progressively higher values which appear at the lower end of the body-size scale are reflections of the impossibly high grazing rates that would be required to supply the food requirements related to their routine metabolism if predators were forced to feed on the vanishingly small biomasses of fish prey that would be under the extreme left-hand limb of the fitted dome.

It is apparent that this extrapolation of the calculations of the values of Kf provides us with an indication of the situation in which predators within the fish dome must obtain their food from the next lower trophic position. That is, with a given range of Kf available from considerations of the physiology and behaviour of the predator, the density of prey organisms needed to satisfy their food requirements must be greater than is available at the smallest sizes in the fish trophic position. In this case, one would expect that predators would find it much more profitable to feed on prey organisms in the next lower trophic position where there must be size categories characterized by a much higher biomass density, albeit at relatively low prey values of log P/B (Boudreau et al. 1991).

TABLE 1. Summary of model parameters found for Chadwick's (1976) lake data.

Parameter	Value	Units
logB_0	-0.45 ± 0.18	log(m^{-2})
C_0	-0.67 ± 0.12	log(m^{-2})/log^2(kcal)
logw_0	-0.81 ± 0.20	log(kcal)
loga'	0.47 ± 0.17	log(yr^{-1})
γ'	-0.29 ± 0.11	log(yr^{-1})/log(kcal)
log$R$	-3.0	
loga''	1.85 ± 0.65	log(yr^{-1})
γ''	-2.29 ± 0.39	log(yr^{-1})/log(kcal)
log$R$	-4.0	
loga''	3.64 ± 1.08	log(yr^{-1})
γ''	-2.96 ± 0.51	log(yr^{-1})/log(kcal)

A comparison of Fig. 4A, where the modeled log$R$ is -3, with Fig. 4B, where log$R$ is -4, shows that as the distance between predator–prey domes is increased (i.e., prey become relatively smaller), the slope of the Kf coefficients with predator body size also increases. This implies that in order for predators to be able to feed on smaller prey, there is a requirement for a more rigorous adaptation of the parameters of predation success in relation to body–size. Such effects may well reflect natural limitations on the abilities of predators to take advantage of higher rates of production by smaller prey unless prey density were to increase in proportion. Such a general prey-size limitation for larger predators may be reflected in the fact noted by Boudreau and Dickie (1992) that while there seems to be relatively little difference among average values of log$R$ among ecosystems, its value appears to increase (prey become relatively larger) as one moves from smaller to larger trophic positions within ecosystems.

Discussion

Because we can identify biomass domes within an aquatic ecosystem as mathematical consequences of the allometric production relationships that emerge from the body-size dependence of various metabolic and trophic parameters, we are in a position to specify a number of properties of the equations for interpretation of the successions of biomass domes observed in nature.

Initially, for example, we note by comparing eq. (8) and (11) that in the multispectral case the horizontal spacing between parabolic dome peaks goes to zero in the special case where the curvature is

$$c = c_0 = \frac{-\gamma}{\log R}.$$

Here the separate domes have merged together to form an aggregate dome that is indistinguishable from the quadratic part of the solution for the biomass spectral density of the single trophic position model introduced in the section entitled Feeding linkages within a trophic position. This conclusion is of significance as a means of illustrating the point that the mathematics alone is an insufficient base for distinguishing the single and the multispectral situations. The distinction between them relies on the reality of the values of the a priori biological parameters used to establish the alternative models. That is, the

existence of a multispectrum structure depends on our capacity to identify parameters of energy flow within trophic positions, such as is illustrated above in the indications of the values of Kf required in order to support feeding links within or between domes.

It may be noted that, in general, the horizontal spacing between the dome peaks can be greater than, equal to, or less than $\log R$, depending on the sign of γ. That is, the mathematics does not make an a priori statement about whether organisms at the peak of a predator dome are feeding on organisms to the left or right of the peak of the prey dome. Considering the many behavioural and morphological feeding adaptations that exist, it is certain that both situations arise in nature when the predator–prey groups are described in terms of their individual body sizes and production efficiencies alone.

A situation of special interest arises where $c \rightarrow -\infty$, in which case the biomass domes would become very narrow, and in the limit correspond to discrete vertical lines in the biomass spectrum. In this line spectrum the spacing between the lines reduces exactly to the average feeding size ratio, $|\log R|$. The ratio of total predator mass to total prey mass would then be $B_{j0}w_{j0}/B_{j0}w_{j0}$. It is conceivable that situations of this sort may be approached in cases of strong selectivity of food types, such as arise with extreme scarcity of food resources or strongly pulsed arctic or aquatic "desert" ecosystems and consequent specialization by the predators. That is, the dome widths may be useful as indices of food supply.

While we have concentrated attention on two domes, termed the prey and their predators, it is obvious that the prey dome may represent predators of a third trophic position or a third feeding group within it. Such a third group will also be described by a parabolic dome of the same curvature as the others (Thiebaux and Dickie 1993). Moreover the horizontal displacement of the third group from its predators is h, the same as between the original groups. The original predator dome can equally well be regarded as prey for a predator dome to its right. Evidently this recurrence of new feeding groups or trophic positions can repeat to the left and right until the primary and top groups are reached, or until body sizes are reached that do not obey the simple premises of our model. The result is a biomass spectral density composed of a sequence of equally spaced identically dome-shaped trophic positions. Given the shape and position of one dome, the shape, position, and biomass of all other domes are determined.

The peaks in the sequence of domes that we envision to comprise an overall spectrum lie on a locus which is itself a parabolic arc. The curvature of this locus at its vertex is γ/h. The sign of the curvature of the locus of peaks in a sequence of normally spaced domes ($h > 0$) is the same as the sign of γ. It should be noted, however, in accord with our earlier arguments concerning the twofold allometric body-size scaling of the production processes in ecosystems, that in the "between-domes" case, we envision the values of the parameters of eq. (10) to be derived from the *primary* scaling. It is our expectation in this case that the value of γ will be close to zero or even a small positive number (Boudreau and Dickie 1992; Thiebaux and Dickie 1993), in keeping with the fact that fittings of primary spectra began with the assumption that they are straight lines, rather than shallow parabolas.

Although the implied sequence of peaks continuing forever on a locus of positive curvature would correspond to infinite biomass, positive curvature should not be ruled out for that reason alone. The model must break down at the extreme ends of the naturally occurring biomass spectrum where the sequence of peaks would be cut off. It should be clear, however, that the locus of peaks is a straight line if and only if $\gamma = 0$, in which case the slope of the locus is given by

$$-\frac{\log a}{\log R}.$$

This mathematical specification of the terms of the biomass structure tempts us to a further speculative interpretation of multiple feeding groups within a trophic position as a state in the development of an ecosystem that began as a single feeding group and has since undergone changes in its feeding habits. For example (again referring to eq. (8) and (11)), if a system were to evolve in time, through some unspecified dynamics, in such a way that the average feeding size ratio, $\log R$, were to decrease (smaller prey were taken) relative to its initial single feeding group value $-\gamma/c$, there would result a disaggregation of the group into multiple feeding groups. It is of potential significance that such disaggregation would appear to arise simply from the ecological or evolutionary embedding of the change in particle size of the preferred food into the predator's feeding behaviour.

It seems reasonable to conclude that these fittings of the biomass spectral model by empirical data encourage further exploration and use of the spectrum as a means of estimating the dynamic parameters governing productivity in observed ecosystems and in understanding the significance of certain of the adaptations displayed by predators and their prey.

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Appendix: Equations for Interval Data

An alternative form of eq. (1) is applicable when biomass data are represented in the form of totals within bins regularly spaced on the $\log_w$ scale. A common example is that of adjacent bin sizes on the w scale being in the ratio of 1:2 (Platt and Denman 1978). For a particular feeding group, we have

$$B(w) = w\frac{dN(w)}{dw}$$

where $N(w)$ = number of organisms of sizes $\leq w$; the derivative of $N(w)$ is assumed to exist. Let k = bin size ratio, i.e., adjacent bin sizes are in the ratio of $1{:}k$ where $k > 1$. If w is the left-hand boundary of a bin, kw is the right-hand boundary.

From the mean value theorem applied to the interval $[w,kw]$, we know there exists some k' between 1 and k such that

$$\frac{dN(k'w)}{dw} = \frac{N(kw) - N(w)}{(k-1)w} .$$

It follows that

$$B(k'w) = k'w\frac{dN(k'w)}{dw} = \left[\frac{k'}{k-1}\right]\Delta N$$

where $\Delta N = N(kw) - N(w)$, the number of organisms in the bin.

With acceptable error, in consideration of the logarithmic scaling, we could assume that $k'w$ occurs at the bin midpoint on the logarithmic scale. Letting $\hat{w} = k'w$, we obtain an estimate of the biomass spectral density for the predator group at $\hat{w}_j$:

$$\text{(A1)} \quad B_j(\hat{w}_j) \approx \left[\frac{\sqrt{k_j}}{k_j - 1}\right]\Delta N_j(\hat{w}_j) .$$

Obviously a similar result holds for the prey group i.

If the same bin size ratio is used for representing both predator and prey feeding groups, the term in square brackets cancels when eq. (A2) is substituted in eq. (1), and we obtain

$$\text{(A2)} \quad P_j(\hat{w}_j) = K_j(\hat{w}_j)\, F_j(\hat{w}_j)\frac{\Delta N_i(\hat{w}_i)}{\Delta N_j(\hat{w}_j)} .$$

In terms of the masses ΔM in the bins, we have

$$\text{(A3)} \quad P_j(\hat{w}_j) = K_j(\hat{w}_j)\, F_j(\hat{w}_j)\frac{\hat{w}_j}{\hat{w}_i}\frac{\Delta M_i(\hat{w}_i)}{\Delta M_j(\hat{w}_j)} .$$

Equations (A2) and (A3) are alternative versions of the basic eq. (1) of particular utility in empirical fittings and visualizations of the relationships by which we describe the dynamics underlying the biomass spectrum.

Strontium Microstructure in Scales of Freshwater and Estuarine Striped Bass (*Morone saxatilis*) Detected by Laser Ablation Mass Spectrometry[1]

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A laser ablation microprobe with mass spectrometry was used to examine the variability of Sr concentration among and within scales of striped bass (*Morone saxatilis*). These exploratory studies demonstrated that the technique is capable of showing differences that we relate to environmental conditions. There were marked differences in scale Sr concentrations at high spatial resolution between striped bass from freshwater reservoirs in Tennessee (low Sr) and the Roanoke River – Albemarle Sound estuary system in North Carolina (high Sr). A peak in Sr concentration was found near the scale focus of striped bass collected in the freshwater Roanoke River (but not in scales from reservoir fish) that may identify saltwater parentage or estuarine rearing. In fish from the Roanoke River and Albemarle Sound, we observed spatial variations across annuli that might reflect movements between fresh and saline water. There was a typical Sr profile with depth through the estuarine scales that exhibited a peak of unresolved significance near the inside surface. Laser ablation mass spectrometry of Sr in fish scales appears to be a valuable technique for life history analyses.

Nous avons utilisé une microsonde à ablation par laser avec la spectrométrie de masse pour examiner la variabilité de la concentration de Sr parmi et dans les écailles du bar rayé (*Morone saxatilis*). Ces travaux exploratoires ont montré que la technique peut révéler des différences qui sont en rapport avec les conditions environnementales. On notait des différences marquées dans les concentrations de Sr dans des écailles à une résolution spatiale élevée entre le bar rayé des réservoirs d'eau douce du Tennessee (faible) et le système estuarien de la rivière Roanoke et de la baie Albemarle, en Caroline du Nord (élevée). On a observé un pic de la concentration de Sr près du foyer de l'écaille des bars rayés capturés en eau douce dans la rivière Roanoke (mais pas dans les écailles des poissons provenant des réservoirs), ce qui peut représenter un héritage de la vie en eau salée ou un élevage en estuaire. Chez les poissons de la Roanoke et de la baie Albemarle, on a observé des variations spatiales en travers des anneaux qui pourraient représenter les déplacements des poissons entre l'eau douce et l'eau salée. On a noté un profil de Sr typique en fonction de la profondeur dans les écailles des poissons estuariens qui révélait un pic, dont la signification reste obscure, près de la surface intérieure. L'emploi de la microsonde à ablation par laser et de la spectrométrie de masse pour étudier Sr dans les écailles de poisson semble une méthode précieuse pour l'analyse du cycle biologique.

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Chemical analyses conducted within small portions of fish otoliths through the use of a wavelength dispersive microprobe technique have proven useful for distinguishing life history patterns (Kalish 1989, 1990). The technique can distinguish between periods of freshwater (low Sr/Ca ratio) and marine (high Sr/Ca ratio) residence and also whether fish have originated from anadromous or freshwater parents when both types exist within a species (Kalish 1990). For the latter determination, high Sr/Ca ratios in otolith primordia, or nuclei, reflected elevated Sr content of the saline water in which parental vitellogenesis, or yolk formation, took place. Sr can chemically substitute for Ca due to structural similarity (Skoryna 1981); the relative degree of substitution depends largely on the relative concentrations of Sr and Ca in the environment. Sr/Ca ratios in seawater are typically 0.0087 (Bruland 1983) whereas they average 0.0019 in freshwater (Rosenthal et al. 1970). In an earlier study, Kalish (1989) related natural Sr in water to Sr in endolymph and otoliths of brown trout (*Salmo trutta*).

There would be immense opportunities for environmental sciences if the types of life history information obtained by Kalish (1990) using Sr could also be obtained with other elements and hard parts and applied to other fish management situations. Fisheries managers would like to be able, for example, to identify the origin of wild stocks of anadromous fishes by trace elements in the scale foci derived from natal streams, to clearly distinguish all hatchery-reared fish by trace element marks laid down as fry, or to precisely determine the duration of residence of migratory fish in specific habitats characterized

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by unique water chemistry. The wavelength dispersive microprobe, however, has a limited repertoire of chemical elements that are reliably quantified (Coutant 1990; Kalish 1990). The small number of suitable elements restricts the variety of potential applications of this type of microprobe chemical analyses for retrospective analyses of habitat use.

Our study sought to demonstrate whether another type of microprobe chemical analysis that potentially has a more diverse chemical repertoire, laser ablation mass spectrometry (Arrowsmith 1987), could also identify differences in relative Sr content within fish hard parts that would be useful indicators of life histories. If so, then further studies using the technique with Sr and other elements might be warranted. Laser ablation techniques have been used by physical scientists for individual small particle chemical analyses (Kolaitis et al. 1989) but only rarely for analyzing biological samples (Heinrich et al. 1986; Benyaev and Arefiev 1992). Laser techniques have a number of theoretical advantages over electron probe methods (Coutant 1990).

For this exploratory study, we used the scales of striped bass (*Morone saxatilis*). We see many reasons why managers of striped bass populations might want to have detailed chemical markers of habitat use such as those identified by Kalish (1990). In striped bass, as in salmonids, the ability to differentiate between fish originating or rearing in marine or freshwater, the ages when fish make the transition between saline and freshwater habitats, and the degree to which large adults move between riverine and coastal waters may be important for management of local populations. Several coastal systems, for example, the Roanoke River – Albemarle Sound, North Carolina, the St. Johns River, Florida, and the Appalachicola River, Florida, have reproducing populations of striped bass in both upstream reservoir–river systems and in downstream estuaries and coastal waters. In these systems, the source of lower river residents and estuarine spawning adults is currently unknown. Striped bass populations south of Chesapeake Bay tend to be increasingly riverine, and the river-resident fish rather than coastal migrants may dominate a spawning stock. Also, extensive stocking of freshwater-reared striped bass has occurred recently in coastal waters to augment declining natural estuarine reproduction. It is important for assessment of both natural reproduction and the effectiveness of stocking programs to be able to distinguish hatchery stocks with different lengths of initial freshwater rearing from natural estuarine fish in later catches. Regulatory agencies charged with distinguishing striped bass in the marketplace as products of coastal and freshwater fisheries or aquaculture need a reliable assay method (Belanger et al. 1987).

For some purposes, conventional wet-chemical methods that analyze whole or dissected portions of scales, otoliths, or other hard parts may give sufficient information whereas other applications need to distinguish Sr or other elements in highly localized areas. Often, it is the length of time spent in one habitat or another that managers would want to distinguish. For this reason, microprobe techniques that relate chemical content to age-specific layers of hard parts are particularly attractive. In our study, we explored a level of spatial detail that would allow us to evaluate whether the technique could give that resolution.

Scales may be more useful than otoliths as a medium for identifying life history events in many circumstances. Scales can be removed without killing the fish. Thus, samples can be obtained from, for example, brood stock that must be kept alive, commercial or sport catches without defacing the product, or individuals from rare and endangered stocks that must be preserved. The ease with which scales can be removed facilitates routine archiving of samples when fish are collected for nearly any other purpose. Scales also do not require the extensive mounting and grinding typical of otolith preparation. For these reasons, we sought to demonstrate the efficacy of analyzing scales rather than otoliths for differences in strontium content related to salinity. However, loss, resorption, and regeneration of scales during ontogeny can occur (Bilton 1974) and must be taken into account when scale samples appropriate for analysis are chosen.

Use of microprobe chemical analysis techniques, in which distinct growth zones of scales can be analyzed separately, seems especially suitable for resolving the equivocal status of existing scale data for discrimination of salinity histories. Bagenal et al. (1973) distinguished between freshwater resident brown trout and sea trout on the basis of Sr content of whole scales. Moreau and Barbeau (1979) did likewise for lake whitefish (*Coregonus clupeaformis*). Unreliable results were obtained, however, by Castonguay and FitzGerald (1982) and Gausen and Berg (1988) who attempted to distinguish between anadromous and nonanadromous brook trout (*Salvelinus fontinalis*) and Atlantic salmon (*Salmo salar*) using conventional techniques.

We had two main objectives for this study: (1) to test the capability of the laser ablation mass spectrometry technique for elemental analyses of fish scales and (2) to learn some useful information about Sr microdistribution in scales. For the second main objective, we had four subobjectives. First, we sought to discern notable differences, indicative of salinity differences, in Sr concentrations in many small areas of striped bass scales from freshwater reservoirs in Tennessee and the Roanoke River – Albemarle Sound estuary system in North Carolina. Second, we wanted to know if we could show high Sr concentrations in scale foci of striped bass collected in the Roanoke River and its estuary that might be used to identify saltwater parentage similar to that documented by Kalish (1990) for salmonids. Third, in fish from the Albemarle Sound system, we wished to see if the technique would distinguish spatial variations in relative Sr concentration across scale annuli that might reflect movements between fresh and saline water. And fourth, because the laser ablation technique can sample deep in tissues as material is progressively ablated (vaporized), we wanted to see if there were Sr differences through the thickness of the scales.

Methods

Striped bass were collected by various methods from three freshwater reservoirs in eastern Tennessee (Cherokee, Watts Bar, and Norris) which are far removed from coastal influences, from the freshwater Roanoke River at Weldon and Plymouth, North Carolina, that is confluent with Albemarle Sound, North Carolina, and from two sites farther into the brackish Albemarle Sound. Reservoir samples came from field studies by the first author and his students (Coutant 1985). Roanoke River and Albemarle Sound samples were provided by J.W. Kornegay of the North Carolina Wildlife Resources Commission. Most of the fish for which scales were examined were fairly large and some were probably sexually mature (Table 1). Scales were removed from the fish with forceps or scalpel and placed into manila paper envelopes where they dried and remained several

TABLE 1. Sampling locations and lengths and weights of striped bass analyzed with laser microprobe for Sr content in their scales.

Collection site	Total length (cm)	Weight (kg)
Freshwater reservoirs, Tennessee		
Norris	85.0	7.7
Norris	68.8	4.6
Norris	81.0	5.9
Cherokee	75.0	6.7
Cherokee	79.0	5.7
Cherokee	81.5	6.2
Cherokee	95.4	9.1
Watts Bar	90.0	7.9
Watts Bar	101.2	13.5
Watts Bar	84.8	6.1
Roanoke River – Albemarle Sound, North Carolina		
Roanoke River at Weldon	81.7	8.0
Roanoke River at Weldon	77.1	6.1
Roanoke River at Weldon	77.5	7.0
Roanoke River at Weldon	41.0	1.9
Roanoke River at Weldon	76.7	6.7
Roanoke River at Weldon	78.5	6.2
Roanoke River at Plymouth	42.4	0.9
Pasquotank River	33.9	0.4
Pasquotank River	39.1	0.6
Albemarle Sound	33.6	0.4

years until our use. Prior to mounting for analysis, scales were separated by soaking in distilled water and cleaned manually.

The scales were mounted on planchets and placed in a stainless steel vacuum chamber. A Xe-Cl Lumonic excimer laser was used for the ablation (vaporization of minute amounts of the target material by the burst of energy from the laser). The laser beam pulse duration was measured ~15 ns. The laser energy for each pulse was monitored by a photodiode detector that was well calibrated by a Coherent 210 power meter. The typical laser energy per pulse was about 18 ± 2 mJ. The ablation angle was 45°. The laser beam distribution was fairly close to a square wave and was typically focused to a diameter of about 0.4 mm on the target. The repetition rate of the ablation laser was set at 0.5 Hz to prevent local heating of the samples. The ablated material from each laser pulse was analyzed separately.

The ions produced by ablation were selected by a quadrupole mass filter and subsequently detected by a channeltron multiplier. The transmission curve of the quadrupole mass filter was obtained by putting various gas molecules with known electron impact ionization through the device. The overall desorbed ions were collected by a parallel-plate arrangement. Since the number of the total desorbed ions could be measured and the percentage of each selected ion could be obtained from a well-calibrated curve, a quantitative determination of selected desorbed ions was possible. A calibrated charge sensitive preamplifier was used and the output was displayed on an oscilloscope or went into a time-shaping amplifier. A sample-and-hold electronic circuit was built to measure the result of each laser pulse. We did not attempt to determine absolute concentrations of Sr per unit of scale material but instead used a relative scale per laser pulse. The digitized output was fed into a microcomputer for simple data analysis (e.g., all data from a single scale or scale source could be averaged).

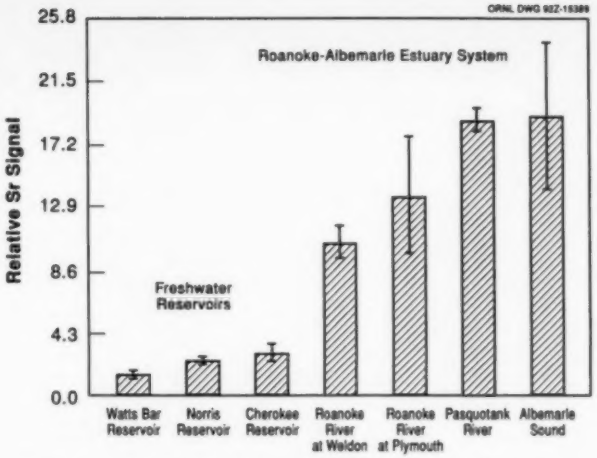

FIG. 1. Relative Sr signal strengths in scales from three Tennessee freshwater reservoirs (Watts Bar, Norris, and Cherokee) and four sites in the Roanoke River – Albemarle Sound estuary system (lower Roanoke River at Weldon and Plymouth, Pasquotank River, and Albemarle Sound) (average and range of depth-averaged Sr at all spots on all fish scales from a collection site). Numbers of fish are given in Table 1.

For the objective of broadly comparing freshwater and estuarine fish scales, we sampled several representative spots across the surface of several scales of different striped bass from each location. Sr values from individual laser pulses were recorded as the laser ablated material from the surface to progressively deeper layers, and an average Sr value for a spot was calculated. We then calculated the average and range of all of these spot-specific values by fish collection site. For the objective of seeking possible high Sr in the scale foci, we took transects of spots from the focus to the outer edge of several scales, averaging 10 or more laser pulses at each scale location. These transects also gave us information for another objective, that of determining if there were horizontal differences that might represent movements between fresh and salt water. For some locations on representative scales, we also recorded and plotted the Sr values for individual laser pulses as the ablation process progressed through the scale from one scale surface to the other. Unlike Kalish (1990), we used Sr concentration alone and not as a Sr/Ca ratio. This selection was arbitrary and presumes that the Ca of scales is rather uniform throughout.

Results

Laser ablation sampling of representative spots on many fish scales showed notable differences among averages and ranges of Sr concentration in scales from freshwater reservoirs of Tennessee and the entire Roanoke River – Albemarle Sound estuarine system (Fig. 1). There was no overlap between depth-averaged reservoir (none above 4 on our relative scale of concentrations) and estuarine (all above about 9) Sr concentrations, which differed by a factor of about 7. Sr varied little within or among freshwater scales compared with estuarine samples, for which relative concentrations varied by a factor of nearly 3. Of the freshwater sites, the highest Sr values and most variation among samples occurred in Cherokee Reservoir, which was the most eutrophic as a result of anthropogenic discharges.

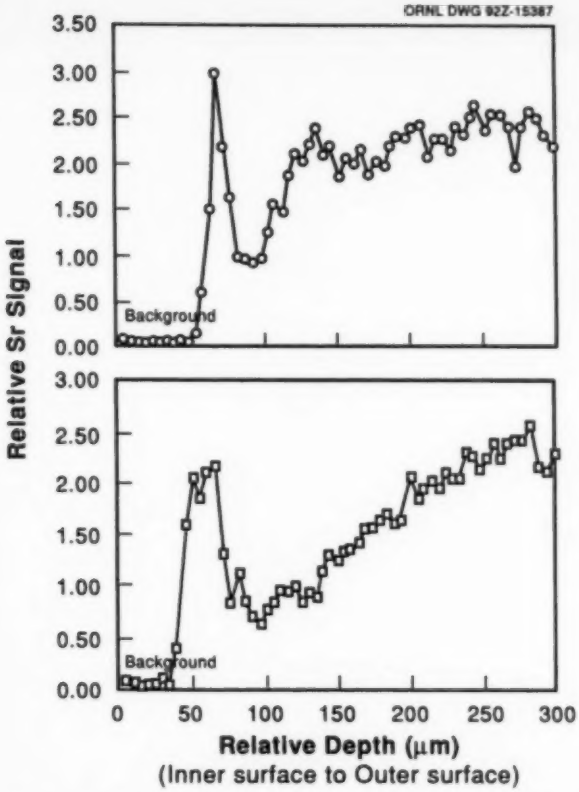

FIG. 2. Two typical examples of relative Sr signals in profiles through the thickness of striped bass scales from the Roanoke River – Albemarle Sound estuary system. Several background measurements are shown at left followed (left to right) by the concave (inner) surface and the remainder of the scale. The depth axis is the focal point of the laser beam relative to an arbitrary starting point above the fish scale; depths through scales are relative and related to the ablation of scale material with successive laser pulses. The peak at the 50–70 μm depth was not seen in freshwater scales.

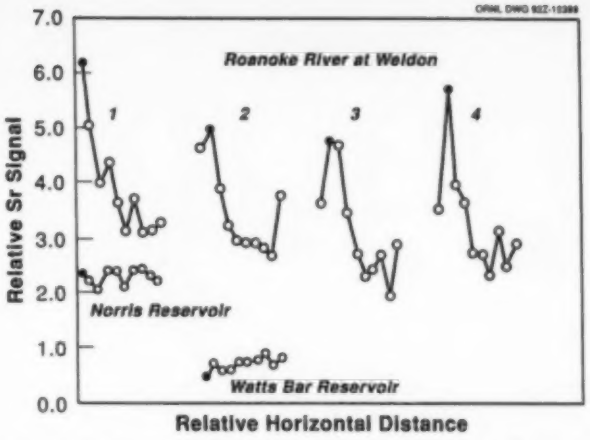

FIG. 3. Horizontal variations in relative Sr signals in typical striped bass scales from the Roanoke River at Weldon (four replicates from one scale) and two freshwater reservoirs. Each fish scale was sampled from the scale focus (solid circle at left) outward to about the first annulus. Initial samples on replicates 2–4 missed hitting the focus precisely. Horizontal (radial) dimensions are only relative because the laser beam movement was uncalibrated.

We also detected variations in Sr concentrations through different parts of the scales. We quickly observed that there were variations through the thickness of scales (Fig. 2) and along the radius of a scale (Fig. 3). As the ablation process at a specific spot removed progressively more material, by boring a hole into and eventually through the scale, a consistent pattern of Sr content with depth was revealed in the scales from estuarine striped bass (but not from the ones from freshwater reservoirs). A peak concentration nearly always occurred near the surface of the concave (inner) side of an estuarine scale (Fig. 2). This peak of Sr was sometimes surpassed by elevated Sr in deeper layers, but it nonetheless stood out as a characteristic feature of material ablated from near the surface. There was not a comparable peak near the convex (outer) side. Scales from reservoir fish simply showed a rise in Sr from background to a level that remained quite constant.

The samples from estuarine striped bass, including those of fish collected from the freshwater Roanoke River, showed elevated Sr at scale foci. When the laser microprobe was aimed first on the scale focus and then successively at spots along a scale radius, there was diminishing Sr farther along the transect (Fig. 3). This pattern was highly repeatable on any one scale, as Fig. 3 shows for four repetitions on a single scale from the Roanoke River at Weldon (the focus was hit imprecisely on three sequences, causing a lower Sr value on the initial sampling). The pattern of high Sr at the focus was not seen in scales of reservoir fish, for which Watts Bar and Norris reservoirs are typical examples (Fig. 3). Following a zone along the radius that was low in Sr, the values in scales from the estuary often rose again with considerable variation.

Discussion

These exploratory studies with laser ablation mass spectrometry raise as many questions as they answer about the origin and meaning of the microstructure of Sr in striped bass scales. Nonetheless, our research has shown that the technique is capable of distinguishing this microstructure and that we should be able to derive meaningful biological conclusions from its use. Success in detecting differences in Sr suggests that other elements may also be examined fruitfully.

The striped bass scales we analyzed at representative spots with a laser microprobe clearly illustrated differences in Sr content between freshwater and saltwater individuals. These differences were found before by Bagenal et al. (1973) and Moreau and Barbeau (1979) who had analyzed the Sr content of whole salmonid scales. For this level of distinction, analysis of whole scales by conventional chemical techniques would be sufficient and probably faster, less expensive, and statistically more reliable. Although our microprobe analyses were able to distinguish several characteristic differences in Sr content at locations within a single scale, none of these constituent differences masked the fundamental dichotomy between Sr content of scales from estuarine and freshwater fish. The relative paucity of Sr in scales taken from striped bass living in reservoirs is consistent with the low ratio of Sr to Ca in freshwater (Rosenthal et al. 1970; Bruland 1983).

It is in the detection of variations in Sr concentration through

different parts of a scale that the technique finds its special application. The horizontal Sr microstructure that we observed in striped bass scales, even at the relatively coarse resolution of 0.4 mm, appears to be useful for deciphering salinity history. The pattern of high Sr content at the focus that declines over a short distance in scales collected from striped bass living in estuaries seems to agree with the findings of Kalish (1990) who used a wavelength dispersive electron microprobe with anadromous salmonids. Typical anadromous striped bass would also have parental vitellogenesis, or yolk formation, take place under conditions of higher Sr/Ca ratio in saline water because much of the mature population resides in marine or estuarine conditions (Coutant 1985). After spawning in freshwater, the larval fish and early juveniles reside in low-salinity waters of rivers and upper estuaries. The explanation of Kalish (1990) that the higher marine Sr is transferred to the egg yolk and then to the developing embryo and fry, even when incubation occurs in freshwater, would be consistent with our observations and the striped bass life cycle. Alderdice (1988) explained how Ca (and therefore, Sr) is important for ionic regulation in teleost eggs.

It is somewhat surprising, however, that the maternal Sr signal would be so pronounced after transfer through the small-diameter striped bass egg and that it remains long enough to be incorporated in the scales that begin to form well after complete utilization of the yolk. An alternative explanation for our high Sr near the scale focus could be high Sr uptake during early estuarine rearing of striped bass juveniles when scale formation is initiated. A higher early Sr uptake might also result from feeding lower on the food chain before piscivory begins. More information, particularly a controlled laboratory study, would be needed before our exploratory data can be interpreted with confidence.

Whatever the mechanism, occurrence of high Sr in the focus of scales from the Roanoke River at Weldon was detectable with the laser ablation technique and it indicates that fish collected as adults from this freshwater environment were most likely derived from the coast or brackish estuary. Had these fish originated from stocks in upstream freshwater reservoirs, where striped bass have self-reproducing populations, the Sr content at the focus would have been low and more similar to the content in scales of fish from the Tennessee reservoirs. It has been suspected that adult striped bass may be year-round residents in the freshwater lower Roanoke River due particularly to avoidance of warm temperatures in summer in Albemarle Sound. Such year-round riverine residents might be expected to have Sr concentrations more like fish from freshwater reservoirs. Additional quantitative analysis will be required before we can confidently establish the history of the Roanoke River fish. The laser ablation technique, with its ability to probe just the focal area of a scale, appears capable of resolving the question.

In principle, the record of a fish's movements in and out of saline water should be distinguishable by tracking variations in Sr content along scale radii. Differences in Sr content could be associated with annuli and specific interannulus locations to pinpoint the time of year and the fish's age when such movements take place. Such a record was demonstrated by Kalish (1990) in salmonid otoliths for which the life history was known. Although we have observed Sr variations along scale radii, we have not yet combined the laser microprobe with age analysis, which would have to be done on the same fish scale. The laser ablation technique leaves a small hole in the scale that can be seen easily under magnification and associated with growth marks. If the low probe resolution that we used in this study is improved (a laser focus on the order of 20 μm is possible), then a more detailed chronology could be obtained. This improvement will require a reduction of focal length of the laser and necessitate modification of our vacuum chamber.

We currently have no reliable explanation for the characteristic peak of Sr near the concave surface that is seen in vertical profiles through our scales. Because we did not analyze Ca concurrently, the zone of high Sr may be a zone of higher calcification. The peak may be a manifestation in the vertical plane of the higher Sr seen near the focus in horizontal transects. Vertical differences in Sr concentration (either alone or in relationship to Ca) will have to be taken into account when horizontal differences are examined for evidence of changing environmental conditions as fish age. This feature will require more examination of both scales and physiological literature.

These exploratory studies add support for the use of scales for obtaining detailed life history information from chemicals deposited during growth. Whereas otoliths are generally considered the medium of choice for the most accurate life history dating and microprobe analyses (Kalish 1990), scales have advantages for both the fish and the investigator. To be able to read the important aspects of life history that we have seen here without sacrificing the fish or necessitating extensive tissue preparation should be a stimulus to broader use of the technique.

The exploratory work also supports the notion that understanding the fine structure of scale chemistry can be a key to clearly resolving environmental history. Analysis of whole scales, as was used in several earlier studies, can logically lead to ambiguous or contradictory results. Variations in elemental concentrations within a scale caused by parentage and individual environmental exposures can confuse the interpretation of integrated lifetime exposures determined from analysis of whole scales. The fine structure of one characteristic element (Sr) that we used appears to be more reliable for determining salinity history than was the gross content of many chemicals in whole scales, even when discriminant analysis is used with multiple elements (Belanger et al. 1987). Because the localized content of Sr in scales from striped bass in the estuarine system varied from near the levels in known freshwater fish to levels about 7 times higher, we feel confident that the ablation method can be used to identify the times in a fish's life when it has moved from freshwater to seawater and vice versa.

The use of Sr alone and not as a Sr/Ca ratio, on the assumption that Ca is relatively constant, may not be valid, and the question deserves additional study. The laser ablation technique we used is suitable for multiple-element analyses, however. It has the capability of measuring many more than the six elements (Ca, Na, Sr, K, S, Cl) that Kalish (1990) concluded can be reliably measured using wavelength dispersive electron microprobes.

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Relationship of Salmonine Production to Lake Trophic Status and Temperature[1]

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Plante, C., and J.A. Downing. 1993. Relationship of salmonine production to lake trophic status and temperature. Can. J. Fish. Aquat. Sci. 50: 1324–1328.

Data on trout, char, and salmon from lakes in several geographic areas indicate that salmonine production (P, kilograms per hectare per year) increases with total phosphorus concentration (TP, micrograms per litre) as $\log_{10}P = 0.47 + 0.95 \log_{10}TP$ ($r^2 = 0.61$). A positive relationship was also found between P and phytoplankton productivity and this relationship suggests that energy transfer efficiencies from phytoplankton to salmonines are reduced in eutrophic lakes. Lake area and mean depth had no significant statistical effect on P but salmonine production was significantly lower in warmer climates. Analysis of these data suggests that projected global increases in air temperatures could lead to about 50% reductions in salmonine production and yield in the north temperate zone.

On a tiré de la littérature des estimés de production (P, kilogrammes par hectare par année) de populations de truites, d'ombles et de saumons dans des lacs de différentes régions. Ces données indiquent que la production des salmoninés augmente avec la concentration en phosphore total (TP, microgrammes par litre) selon l'équation suivante : $\log_{10}P = 0{,}47 + 0{,}95 \log_{10}TP$ ($r^2 = 0{,}61$). Une relation positive entre P et la production du phytoplancton est aussi révélée par l'analyse des données et suggère que le transfert d'énergie entre le phytoplancton et les salmoninés est réduit dans les lacs eutrophes. La superficie et la profondeur du lac n'ont pas d'effets statistiques significatifs. Par contre, on trouve une corrélation positive significative entre P et la température de l'air. Suivant ces derniers résultats, une réduction d'environ 50 % de la production des salmoninés pourrait résulter du réchauffement projeté du climat.

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Evidence is accumulating that nutrients ultimately control the rate of fish production in lakes (Eggers et al. 1978; Mills 1985; McQueen et al. 1986; Mills et al. 1987; Stockner 1987; Downing et al. 1990), especially in lakes with short trophic pathways (Stockner 1987). It has been frequently observed that increases in anthropogenic phosphorus and nitrogen loading to lakes increase their general productivity (see Vollenweider 1974). Fertilizer has been used to maintain high rates of production in aquaculture (Moav et al. 1977; Knud-Hansen et al. 1991). Some have attempted, therefore, to formulate equations to predict fish yield (Hrbáček 1969), fish biomass (Hanson and Leggett 1982), or fish community production (Downing et al. 1990) in lakes using variables related to autotrophic producers, such as total phosphorus concentration or primary production. Models to predict the production of species, or groups of species, unfortunately usually require knowledge of the standing biomass of fish populations (Downing and Plante 1993), data that are costly to obtain.

Because salmonine fisheries are of special economic importance, the prospect of enhancing their production through nutrient addition is appealing. Indeed, the idea that phosphorus is the primary limiting nutrient in lake ecosystems (Wetzel 1983), and the observation that the growth of fish increases with food supply (Brocksen et al. 1970), has prompted some to experiment with phosphorus addition to oligotrophic lakes as a means of enhancing the production of salmon fisheries (LeBrasseur et al. 1978; Hyatt and Stockner 1985; Stockner 1987). The success of these programs (Stockner 1987) suggests that lake fertilization may be a generally applicable method of increasing the quality of recreational and commercial salmonine fisheries. This approach may not be generally applicable, however, considering that several other factors such as recruitment (Colby et al. 1972; Burgner 1987), allochthonous energy inputs (e.g., Hartman and Burgner 1972), and fish community structure (Hatch and Webster 1961; Colby et al. 1972; Hartman and Burgner 1972; O'Connor and Power 1973) are known to limit salmonine populations. Recently, Stockner (1987) has suggested that analysis of a broad range of data on salmonine production measurements might reveal general empirical relationships between trophic status and productivity. The purpose of this study is to test the hypothesis that there is a general positive relationship between lake trophic status and salmon, char, and trout production. In addition, we test the hypothesis that salmonine production is correlated with lake morphometry and climate.

Methods

Data on the annual production of salmonine populations were obtained from the published literature. Estimates of production were usually calculated using the Allen curve and instantaneous growth methods (Downing 1984). Because we sought data

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TABLE 1. Data on the annual production of trout, char, and salmon populations (P, kg·ha^{-1}·yr^{-1}), total phosphorus concentration in the water column (TP, μg·L^{-1}), phytoplankton productivity (PP, g C·m^{-2}·yr^{-1}), mean depth (Z, m), lake area (A, ha), and annual mean air temperature (T, °C). Fish production values include all ages in Char Lake and Øvre Heimdalsvatn, 0+ to 2+ juvenile salmon in Lake Washington and Dalnee, 1+ trout and older in the Turkey Lakes and Wishart Lake, and ages 0 to 5+ for Loch Leven. Data sources: Efford (1972); Krogius et al. (1972); Morgan (1972); Rigler (1972, 1975, 1978); Wali et al. (1972); Hall and Hyatt (1974); Thorpe (1974); Kloster (1978); Lien (1978, 1981); Tangen and Brettum (1978); Kelso (1985); J.R.M. Kelso (Department of Fisheries and Oceans, Sault Ste. Marie, personal communication); Lam et al. (1986).

Lake	Population	P	TP	PP	Z	A	T
Big Turkey	*Salvelinus fontinalis*	0.6	4.2	1.7	3.4	52	5
Char	*Salvelinus alpinus*	2	3.8	4.1	10.2	53	−9
Dalnee	*Oncorhynchus nerka*	350	91.5	487	31.5	136	2
Dalnee	*Salvelinus malma*	26	91.5	487	31.5	136	2
Little Turkey	*Salvelinus fontinalis*	1.2	6.2	1.4	6	19	5
Loch Leven	*Salmo trutta*	17.5	95	354.1	4	1330	9
Marion	*Oncorhynchus nerka*	11.6	245.8	8	2.4	13	9
Øvre Heimdalsvatn	*Salmo trutta*	13.3	14.7	11.5	4.7	78	2
Washington	*Oncorhynchus nerka*	5.6	34.8	157	33	8760	11
Wishart	*Salvelinus fontinalis*	1.2	5.1	0.7	2.2	19	5

reflecting equilibrium conditions, we omitted from consideration populations supported by stocking (e.g. Hatch and Webster 1961; Miura et al. 1976), and populations intentionally overexploited (Langeland 1986). Annual average phosphorus concentration in the water column (TP, micrograms per litre), annual phytoplankton production (PP, grams carbon per square metre per year), and lake morphometry data such as mean depth (Z, metres) and area (A, hectares) were found in the literature or, in some cases, were obtained through direct communication with the authors of production studies. Annual mean air temperature (T, degrees Celsius; Wernstedt 1972), which is closely correlated with surface water temperature (Straškraba 1980), was used instead of water temperature (see also Schlesinger and Regier 1982) to examine the effects of climate on fish production.

Analyses of the relationships between production of salmonine populations, total phosphorus concentration, phytoplankton production, temperature, and lake morphometric characteristics were performed using simple and multiple regression analyses (Draper and Smith 1982) after logarithmic transformation of all variables to stabilize the variances, linearize responses, and normalize residuals.

Results and Discussion

Production data were obtained for 10 salmonine populations from nine lakes in diverse geographical areas, representing a wide range of trophic status. Lakes included a range of trophic states from oligotrophic to eutrophic (Wetzel 1983) (Table 1). Salmonine production estimates were obtained for lakes ranging from 47°N to the high Arctic, in North America, Europe, and Asia. Populations were comprised of trout, char, juvenile salmon, and land-locked salmon (Table 1).

Total Phosphorus and Phytoplankton Production

Salmonine production (P) was significantly correlated (r^2 = 0.61) with total phosphorus concentration (TP; Fig. 1). This correlation is similar to that found for complete fish community production by Downing et al. (1990). Such a correlation for individual salmonine populations is surprising because correlations between lake trophic status and population production

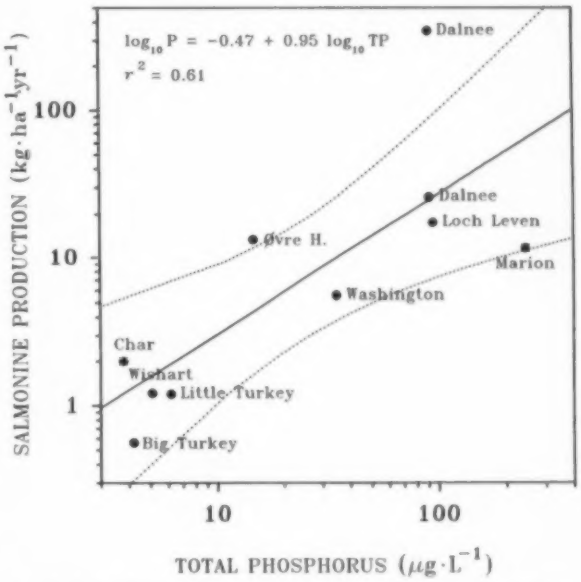

FIG. 1. Relationship between annual production of salmonine populations and total phosphorus concentrations in lakes. The solid line represents the least squares regression equation and the broken lines represent the 95% confidence intervals of predicted means. The residual mean square of the regression equation is 0.30. Lake names appear near each observation.

in other taxonomic groups are rarely very strong unless allowance is first made for the biomass of the fish populations under consideration (Downing and Plante 1993). The link between factors influencing primary production and salmonine populations may be stronger than that for other species because food chains tend to be simple in lakes in which salmonines dominate. The fish populations in the lakes examined here consist mainly of planktivores and benthivores. According to Fig. 1, an increase in total phosphorus from 10 to 100 μg·L^{-1} corresponds to about a 10-fold increase in salmonine production.

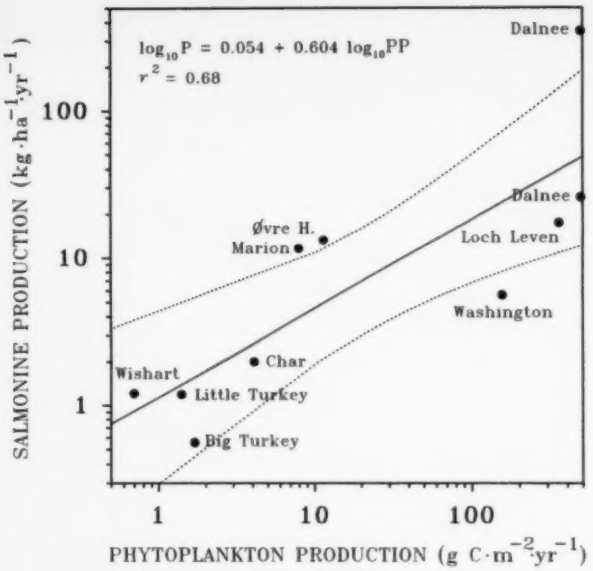

FIG. 2. Relationship between annual production of salmonine populations and the annual phytoplankton production in lakes. The solid line represents the least squares regression and the broken lines represent the 95% confidence intervals of predicted means. The residual mean square of the regression equation is 0.25. Lake names appear near each observation.

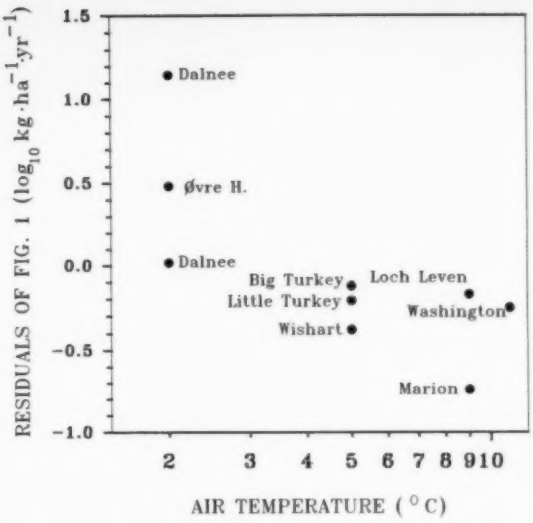

FIG. 3. Relationship between the residuals of Fig. 1 and the annual mean air temperature, excluding Char Lake (see text). Lake names appear near each observation.

Phytoplankton production, closely correlated with total phosphorus concentration (e.g., Brylinsky 1980), is also well correlated with salmonine production ($r^2 = 0.68$; Fig. 2). Such a strong correlation between phytoplankton production and fish production, regardless of fish standing biomass or lake community structure, underscores the suggestion that lake ecosystems can be controlled by primary producers (McQueen et al. 1986). Analysis of the relationship in Fig. 2 (assuming 1 g C = 10 g fresh mass; Oglesby 1977), however, suggests that the conversion efficiency of phytoplankton into salmonine production declines radically from about 1% transfer efficiency in ultraoligotrophic lakes to <0.2% in eutrophic lakes. These results agree with those of Downing et al. (1990) who found that ratios of fish community production to primary production fall as low as 0.002% in hypereutrophic lakes. Such decreases in the transfer of primary production to fish may be due, in part, to the fact that the fraction of phytoplankton biomass that can be ingested by zooplankton declines rapidly above 30 μg TP·L^{-1} (Watson et al. 1992). Our analysis suggests that the transfer of energy from phytoplankton to salmonine production is 10 times more efficient in oligotrophic ecosystems than in eutrophic ones.

Our results should not be interpreted to suggest that there can be unlimited increases in salmonine production under eutrophic conditions. Several studies of the effect of eutrophication on salmonid populations were made during the 1970's (see Colby et al. 1972). These studies suggest that at the onset of eutrophication, salmonid growth generally increases, but the progressive alteration of the spawning areas and competition with other species better adapted to mesotrophic lakes tend to decrease the abundance of salmonid populations over the long term. Equations relating salmonine production and phosphorus or phytoplankton production (e.g., Fig. 1 and 2) probably indicate the potential production of salmonine populations in a lake of a given trophic status. Whether or not this production could be achieved by a salmonine population over long periods depends on other factors influencing the populations. If nutrient addition is to be used as a salmonine production enhancement or management tool, Fig. 1 and 2 represent the potential salmonine production but may not always predict long-term changes in salmonine population production following eutrophication.

Other Factors Related to Salmonine Production

Although it has been suggested that shallow lakes generally support more productive fisheries (e.g., Matuszek 1978; Schlesinger and Regier 1982) and that indices of fish productivity increase or decrease with lake size (e.g., Hayes and Anthony 1964), we found no such correlations for salmonine populations. Correlation analyses of the residuals from Fig. 1 and 2 show that neither lake area nor mean depth was significantly correlated ($p > 0.05$) with salmonine production after accounting for the effects of total phosphorus ($p = 0.21, 0.81$) or phytoplankton production ($p = 0.13, 0.54$). Within the range of lakes studied here, lake depth and area seem to have little influence on salmonine production. Latitudes of lakes in our study range only from 47 to 74°N and, within this range, even shallow lakes like Wishart or Marion probably have temperatures low enough to support healthy salmonine populations. At lower latitudes where surface waters would be warmer (Straškraba 1980), however, lake morphometry may be an important factor mediating salmonine production.

Trout, salmon, and char are all known to be coldwater species (Scott and Crossman 1973); thus, salmonine production should be expected to decline with increased water temperature. Air temperature is probably closely correlated with water temperature in these lakes except in the extremely cold Char Lake where air temperature would grossly underestimate the

annual mean temperature experienced by the fish. If Char Lake is excluded from the data set, there is a negative correlation ($p = 0.03$) between the residuals of Fig. 1 and annual mean air temperature (Fig. 3). A multiple regression of P as a function of TP and T yields the equation

$$\log_{10}P = 0.20 + 1.11 \log_{10}\mathrm{TP} - 1.43 \log_{10}T$$

which has an improved r^2 (from 0.61 to 0.83) and a greatly reduced residual mean squared error (from 0.30 to 0.16) over the comparable statistics calculated in Fig. 1. This analysis indicates that salmonine production is much reduced in warmer regions. This result implies that global warming would decrease salmonine production. In temperate and cold-temperate countries where salmonine populations are currently found, the two-fold increase in carbon dioxide predicted by 2050 (Nuttle and Linton 1992) is expected to result in an increase in the annual mean temperature of 4–8°C (Washington and Parkinson 1986; Mitchell et al. 1990). Such differences in climate are associated with reductions in salmonine production and yields of around 50% following the trend in Fig. 3. This prediction is particularly grave for northern regions that rely heavily upon salmonines for recreational and economic gains.

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Downstream and Early Marine Migratory Behaviour of Sockeye Salmon (*Oncorhynchus nerka*) Smolts Entering Barkley Sound, Vancouver Island

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Behaviour of sockeye salmon (*Oncorhynchus nerka*) smolts was studied during their migration down the Stamp and Somass rivers and through Alberni Inlet and Barkley Sound, Vancouver Island. Emigration from Great Central Lake was typical of other sockeye lakes: smolt abundance peaked in early May and most smolts left the lake at dusk. Migration down the rivers occurred only during twilight or darkness and most smolts made the 25-km journey to tidal waters within a single night. Those that did not held position in tight schools in the surface waters of deep pools during daylight. During downstream migration, ground speed averaged about 2.5 km/h, and smolts did not appear to be especially vulnerable to predation owing to their short residence time, with few smolts remaining in the river during daylight. Catch rates at purse-seine sampling sites throughout Alberni Inlet and Barkley Sound indicated that sockeye smolts migrated more slowly in tidal waters and were sometimes highly vulnerable to predaceous fish (especially Pacific hake, *Merluccius productus*). Estimated migration speeds through Alberni Inlet were 1.9 km/d for smolts entering tidal waters during April and May, and 1.3 km/d for those during June and July; speed through Barkley Sound was 1.6 km/d throughout May–July.

Nous avons étudié le comportement des smolts de saumon rouge (*Oncorhynchus nerka*) pendant leur avalaison dans les rivières Stamp et Somass, puis dans l'inlet Alberni et la baie Barkley, près de l'île de Vancouver. L'émigration du Grand lac Central était typique d'autres lacs à saumon rouge : l'abondance des smolts atteignait un pic au début de mai, et la plupart quittaient le lac au crépuscule. L'avalaison se produisait seulement dans la pénombre ou l'obscurité, et la plupart des smolts parcouraient les 25 km de leur voyage jusqu'aux eaux à marée en une seule nuit. Ceux qui ne migraient pas formaient des bancs serrés dans les eaux superficielles des bassins profonds pendant le jour. Pendant l'avalaison la vitesse sol moyenne était de 2,5 km/h environ, et les smolts ne semblaient pas particulièrement vulnérables à la prédation du fait de leur faible temps de résidence dans les rivières; ils étaient peu nombreux à y rester pendant le jour. Les taux de capture aux points d'échantillonnage à la senne à poche, sur l'ensemble de l'inlet Alberni et de la baie Barkley, indiquaient que les smolts de saumon rouge migraient plus lentement dans les eaux à marée et étaient parfois très vulnérables aux poissons prédateurs (notamment le merlu du Pacifique, *Merluccius productus*). On estime les vitesses de migration dans l'inlet Alberni à 1,9 km/d pour les smolts qui pénétraient dans les eaux à marée en avril et en mai, et 1,3 km/d en juin et juillet; dans la baie Barkley, la vitesse était de 1,6 km/d de mai à juillet.

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Considerable effort has been directed towards understanding the seaward migration of anadromous salmonids (see reviews by Thorpe et al. 1985; Groot and Margolis 1991). In particular, much is known about the behaviour and physiological characteristics of sockeye salmon (*Oncorhynchus nerka*) smolts just prior to emigration, and during emigration from "nursery" lakes in which sockeye fry typically spend their first year of life (Burgner 1962; Johnson and Groot 1963; Groot 1965; Hartman et al. 1967; Foerster 1968; Clarke 1989). However, few studies have documented the behaviour of sockeye smolts during both the downstream migration and subsequent period of early sea life, no doubt owing to logistic difficulties.

Early investigators (Foerster 1929; Barnaby 1944) reported that sockeye smolts migrated downstream passively, often facing into the current of the outlet stream. More recently, other investigators (Hartman et al. 1967; Burgner 1991) concluded that the migration downstream is active where water flow is uniform and quiet, but passive where water flow is turbulent. In contrast, sonic tracking studies indicate that Atlantic salmon (*Salmo salar*) smolts migrate downstream passively, and are continually displaced from feeding territories through behavioural interactions (Fried et al. 1978; LaBar et al. 1978; Tytler et al. 1978; Thorpe et al. 1981). Similar studies show that coho salmon (*O. kisutch*) smolts migrate downstream actively in sections of high water velocity but hold position for extended periods in sections of low water velocity (Moser et al. 1991). Burgner (1991) also pointed out in his recent review that it is not known whether sockeye smolts migrate downstream continuously, day and night. If smolts are especially vulnerable to predation during the downstream migration, as often supposed (Hartman et al. 1967; Tytler et al. 1978), they

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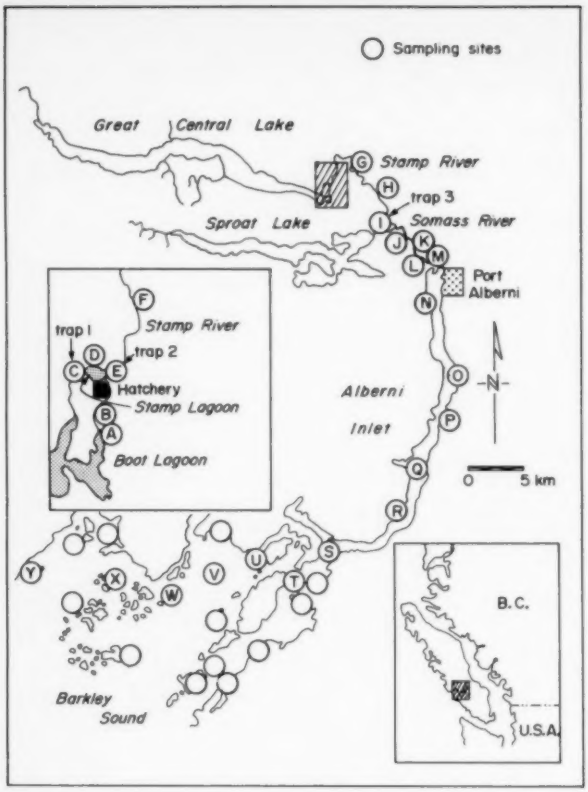

FIG. 1. Map showing locations of sampling sites.

might be expected to seek refugia and hold position in the stream during daylight.

The migration of sockeye during the period of early sea life varies considerably among populations and also depends on latitude (Burgner 1991). Sockeye from southern populations (e.g., the Fraser River) typically enter the ocean in April and May. Groot and Cooke (1987) found that Fraser River sockeye concentrated near the river mouth for a short time, but by the end of May, most had dispersed away from the estuary and were broadly distributed in the Strait of Georgia; the rate of migration during this early period was estimated to be 6–7 km/d. Marine survival of sockeye appears to be controlled by conditions encountered during early sea life (Brett 1986). Predation is widely assumed to be the major source of mortality for all species of salmon during this period (e.g., Parker 1971; Mathews and Buckley 1976; Brett 1986).

To elucidate mechanisms that determine variations in recruitment of Pacific salmon, the Canadian Department of Fisheries and Oceans initiated the Marine Survival of Salmon (MASS) Program. This program has supported a variety of research activities, most focussing on factors relating to the survival of juvenile salmon in Alberni Inlet, Barkley Sound, and along the west coast of Vancouver Island. In this paper, we consolidate new information from these studies concerning the behaviour of sockeye smolts during their seaward migration in a coastal river system and estuary on Vancouver Island. We examine the speed of downstream migration and compare migratory activity during daylight and darkness. We also compare the speed of migration in freshwater with that in tidal waters and comment on the apparent vulnerability of sockeye smolts to predators in these two environments.

Study Area

Sockeye smolts examined in this study emigrated from two Vancouver Island lakes: Great Central Lake and Sproat Lake. Great Central Lake (surface area = 5085 ha) is situated at an elevation of 82 m and is drained principally by the Stamp River (21 km long) but also by Robertson Creek. Robertson Creek flows through the Robertson Creek Hatchery and joins the Stamp River at Stamp Lagoon, a shallow lake about 1 km long (Fig. 1). Sproat Lake (3775 ha) is situated at an elevation of 28 m and is drained by the Sproat River (2.8 km). The Sproat and Stamp rivers converge into the Somass River which flows for another 5.0 km to the sea (Alberni Inlet and Barkley Sound). The zone of tidal influence extends upstream as far as site L in Figure 1. Thus, sockeye smolts from Great Central Lake must migrate a total of 24.9 km at an average gradient of 0.33% before reaching tidal waters. Those from Sproat Lake must migrate 7.8 km at an average gradient of 0.36%. Once in tidal waters, smolts must swim 37 km through Alberni Inlet to reach Barkley Sound and the Pacific Ocean. Since 1987, annual smolt production from Great Central and Sproat lakes has averaged 7 and 9 million smolts, respectively. Most smolts emigrate as yearlings, with annual mean sizes ranging from 3.74 to 6.80 g for Great Central Lake smolts and from 3.50 to 4.74 g for Sproat Lake smolts over the same period (Hyatt et al. 1990).

Methods

Downstream Migration

Fyke nets

Fyke nets (''traps'') were deployed facing upstream in the main current of the Stamp River at three sites to determine daily and seasonal trends in abundance of migrating smolts. The nets measured 2 × 2 m at the upstream opening, were 7.5 m long, and consisted of a graded series of mesh sizes from 5.0 cm at the opening to 1.3 cm at the downstream end. These led via 15-cm-diameter PVC tubing to 1 × 0.75 × 2.2 m collection boxes with baffles to minimize current in the holding chamber. Trap 1 was deployed at site C from 10 to 27 April, trap 2 at site E from 11 April to 9 August, and trap 3 at site I from 13 April to 16 June 1989. Trap 1 was used mainly to estimate the speed of smolt migration across the still waters of Stamp Lagoon by comparing the relative timing of peak catches during the night at the inlet (trap 1) and outlet (trap 2) ends of the Stamp Lagoon. Trap 2 was considered to provide the best index of relative abundance over the season because fishing efficiency at this site appeared to be least influenced by fluctuations in river level.

During the main period of smolt migration (26 April – 12 May), catches were usually recorded (and smolts released) at 20:00, 00:00, 04:00, and 08:00 PDT. At other times, catches were usually recorded between 07:00 and 09:00 PDT. Samples of approximately 50 smolts were collected for size and age composition data from trap 3 each week (except the week of 13–19 May). Catches were also recorded and sampled for size composition (n = 50) at approximately 2-h intervals at all traps on 26–27 April and at traps 2 and 3 on 1–2 and 9–10 May.

To investigate correlations in daily catches at the upstream and downsteam traps, we divided the period of migration into

the "increasing phase" prior to 2 May and a "decreasing phase" after 2 May. We fitted linear trends to the daily catches for each trap in each phase after log transformation to stabilize variances ($r = 0.85$ and 0.95 for traps 2 and 3, respectively, in the increasing phase; $r = -0.91$ and -0.59, respectively, in the decreasing phase). We then removed the linear trends and calculated cross-correlation functions for the residuals at lags of 0–5 d.

Underwater observations

Underwater surveys by divers using SCUBA or snorkels were conducted to observe the diel patterns in migratory behaviour of sockeye smolts. Underwater lights were used during darkness. Divers with SCUBA swam fixed-length transects in Stamp Lagoon (site D, 650 m) and in the Stamp River just above its confluence with the Sproat River (sites I and J, 500 m) at various times of day, 25–27 April, and recorded the number, swimming behaviour, depth, and schooling habits of sockeye smolts. Divers tethered in position and using SCUBA recorded similar observations during 20-min intervals at 20:00, 22:00, 01:30. 03:30, and 05:30 PDT, 9–10 May, both in the section of maximum current and at midchannel in the lower Somass River (site L). Divers using either SCUBA or snorkels also estimated the abundance and behaviour of sockeye smolts during daylight hours at several other sites (G, H, K, and M) where smolts could hold position in the river (i.e., slackwater sections, or deep pools).

Emigration from Great Central Lake

The daily timing of smolt emigration from Great Central Lake was estimated by enumerating smolts against a submerged, white, 1.2 × 2 m flashboard using photographic techniques (e.g., Goodlad et al. 1974), both at the outlet of Boot Lagoon (site A) and in a fast-flowing section of Robertson Creek (site B). The flashboards were photographed (using a flash but no other external light) every minute during 10-min intervals at various times of the day and night from 4 to 18 May. Later, silhouettes of smolts were counted from the photographs.

Physical measurements

Water temperature and level were measured at site E each morning that trap 2 was inspected (usually daily) from 11 April to 20 July. Percent cloud cover and air temperature were also recorded at the same times. On 16 June, we measured maximum river velocity at 0.5-km intervals between sites E and L (40 stations in all), except for a 1-km reach at the Stamp Falls and another 1-km reach of turbulent water on the Somass River. Measurements were taken 20 cm below the surface in the main current of the river using two General Oceanics model 2030 flow meters; at each site, the higher of the two readings was taken as the correct value. River velocity was not measured downstream of site L because there it was obviously influenced by tidal action. The minimum time required for water to flow between sites E and L was calculated by plotting the inverse of the maximum velocity measurement at each station against distance downstream and integrating under the curve.

Marine Migration

The relative abundance and distribution of juvenile sockeye in Alberni Inlet and Barkley Sound were determined by purse seining from the 12.5-m-long vessel RV *KETA*. The purse seine was 275 m long, with a nominal depth of 26 m tapering to 18 m in the bunt. The bunt of the net was constructed of five panels with stretched mesh sizes of 50, 25, 19, 13, and 10 mm. Purse-seine sampling in Alberni Inlet and Barkley Sound began on 13 April and ended on 14 July. From April until mid-May, one purse- seine sample was collected at each of 24 fixed sites every third week. Thereafter, one sample was collected weekly at each site except for a 1-wk gap after the second sample. A total of 192 purse-seine sets were completed in 1989, all during daylight between 06:00 and 21:00 PDT.

All juvenile sockeye caught with the purse seine were immediately identified, counted, and most were released alive. A random subsample of 30–100 fish was retained from each catch; these fish were anesthetized with 2-phenoxyethanol, measured (fork length) to the nearest millimetre, and allowed to recover from the anesthetic before being released alive. The abundance of juvenile sockeye in Alberni Inlet and Barkley Sound is presented as catch-per-unit-effort (CPUE), defined as the total catch in one set of the net at each site.

We estimated the speed of migration of sockeye through Alberni Inlet and Barkley Sound by plotting the dates of peak abundance of juvenile sockeye at 24 marine sampling locations against their respective distances from the first marine sampling location (site N). Dates of peak abundance were determined by inspection of graphical plots and then regressed on distance travelled using several least-squares models (linear, exponential, and polynomial).

Results

Downstream Migration

The downstream migration of sockeye smolts began in mid-April 1989, reached a peak at the beginning of May, and then declined gradually throughout May and June (Fig. 2). Catches in traps 2 and 3 showed similar trends, with peak catches of 1488 and 2887/d occurring on 30 April and 2 May, respectively. Sockeye smolts continued to migrate downstream in small numbers as late as July; the last trap (trap 2) was removed on 20 July when only one smolt was caught. In all, 27 540 sockeye smolts were counted during the sampling period.

Daily catches of sockeye smolts were not correlated with river level, water temperature, cloud cover, or lunar phase in any obvious way (Fig. 2). However, after 10 May, the capture efficiency of trap 3 appeared to increase slightly relative to that of trap 2 because at lower river levels, a greater proportion of the main channel flow was sampled by trap 3 than by trap 2. For this reason, catch data from trap 2 probably gave the most consistent index of relative abundance over the season. These data indicate that 85% of the downstream migration occurred between 23 April and 25 May, during which time daily catches at trap 2 exceeded 200 sockeye smolts.

The average size of smolts declined from 80.5 mm (SD = 7.0) during the first 2 wk of enumeration (period 1, 11–24 April) to 63.7 mm (SD = 5.1) in period 4 (23 May – 5 June) (Fig. 3). Age 1+ smolts accounted for 99.9% of the smolt migration, averaging 2.9 g and 71 mm fork length (range 54–95 mm). Only 6 of 928 specimens collected were age 2+, and these were captured in periods 1 and 2 and ranged in fork length from 85 to 96 mm.

Diel Migratory Behaviour

Cohesive schools of 50–100 smolts were observed at the outlet of Boot Lagoon (site A) in late afternoon between 15:00 and 20:00 PDT (6–10 May), but these resisted the current at the

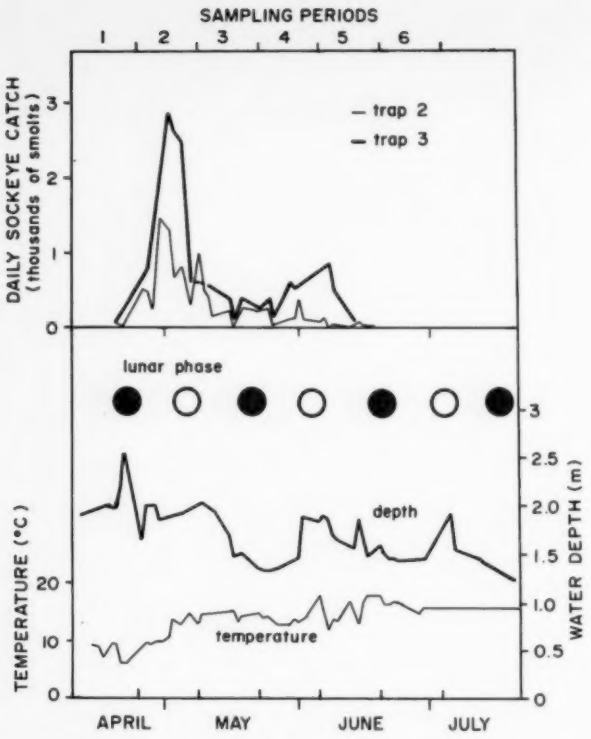

FIG. 2. Seasonal trends in daily fyke-net ("trap") catches of sockeye smolts and water depth and temperature in the Stamp River.

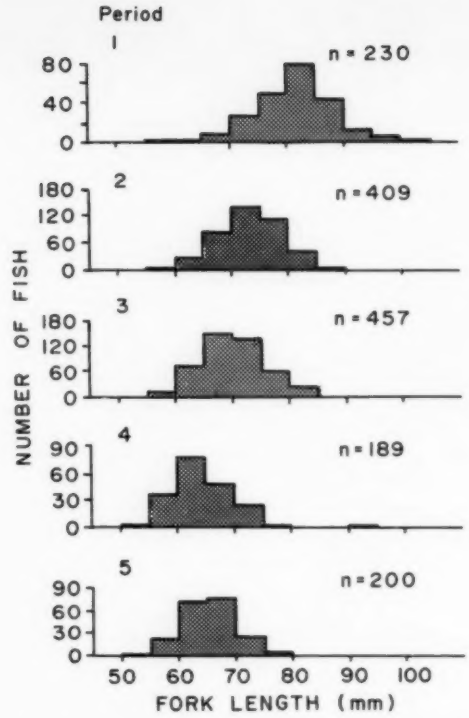

FIG. 3. Length distributions for age 1+ sockeye smolts captured in trap 3 in the lower Stamp River. Sampling periods 1–5 correspond to the 14-d intervals marked in Fig. 2.

outlet and probably remained in the lake until the outlet was in shadow (around 19:00 PDT). Emigration from Boot Lagoon (determined by flashboard counts) reached a peak between 19:00 and 21:00 PDT and had virtually finished for the night by 23:00 PDT. The same pattern was observed 0.5 km downstream in Robertson Creek (site B) where small schools (<50 smolts) were observed to hold position behind small dams in the creek (water control structures for the hatchery) until after 19:00 PDT. Migration down Robertson Creek peaked very early, between 20:00 and 21:00 PDT, and had finished by 23:00 PDT.

Very few sockeye smolts were observed in either the Stamp or Somass River during underwater counts in daylight. The greatest concentration of smolts during daylight was observed at site L, a unique gorge over 12 m deep, with a large, upwelling, back eddy below a highway bridge. About 1000 smolts were frequently observed there, both by divers during scheduled counts and by observers on the bridge at various times of day. These smolts held position in the back eddy in a cohesive school very near the surface. Of the other sites surveyed by divers in daylight (sites D, G, H, I, J, K, and M), all with some slackwater habitat, a few sockeye were observed only at site G (a deep canyon below Stamp Falls) and at site K; these smolts held position in small schools of 2–30 individuals and were often mixed with schools of coho salmon fry.

After dusk, sockeye smolts were observed at all sites surveyed. The large school(s) observed at site L near the surface during daylight disappeared at night and instead, many small groups (<20 smolts) or individuals were observed drifting downstream, most in the zone of maximum current at a depth of 5–8 m. At dawn (05:00 PDT), most smolts at site L were again closer to the surface (1.5–3.0 m depth) drifting downstream in larger schools of up to 50 fish. Although no smolts were observed in Stamp Lagoon (site D) during the day, many smolts were seen there at night, either alone or in loose schools (smolts 0.5–2.0 m apart), and usually in the centre channel of the lagoon, 1–2 m off the bottom (5–6 m depth). When illuminated by diving lights in Stamp Lagoon, smolts remained motionless or else swam quickly to the bottom.

Sockeye smolts at the downstream sampling site (trap 3) were significantly larger ($p < 0.001$, t-test) before midnight (mean fork length = 76.6 mm, SD = 6.4, n = 55) than after midnight (70.5 mm, SD = 6.0, n = 237) on the thee occasions when samples were collected at intervals during the night. In contrast, the mean length of smolts at the upstream sampling site (trap 2) were similar before (69.6 mm, SD = 5.8, n = 77) and after midnight (68.8 mm, SD = 5.8, n = 174) and corresponded closely to the mean length of smolts in trap 3 after midnight; these means were not significantly different ($p > 0.25$, ANOVA). In fact, fewer large smolts (≥80 mm) were captured in trap 2 (5%) than in trap 3 (12%) on these nights ($p < 0.005$, likelihood ratio χ^2).

Speed of Downstream Migration

The nightly pattern of downstream migration is revealed clearly when estimates of relative abundance from photographic records, dive observations, and trap catches are considered together and plotted against time of day (Fig. 4). The

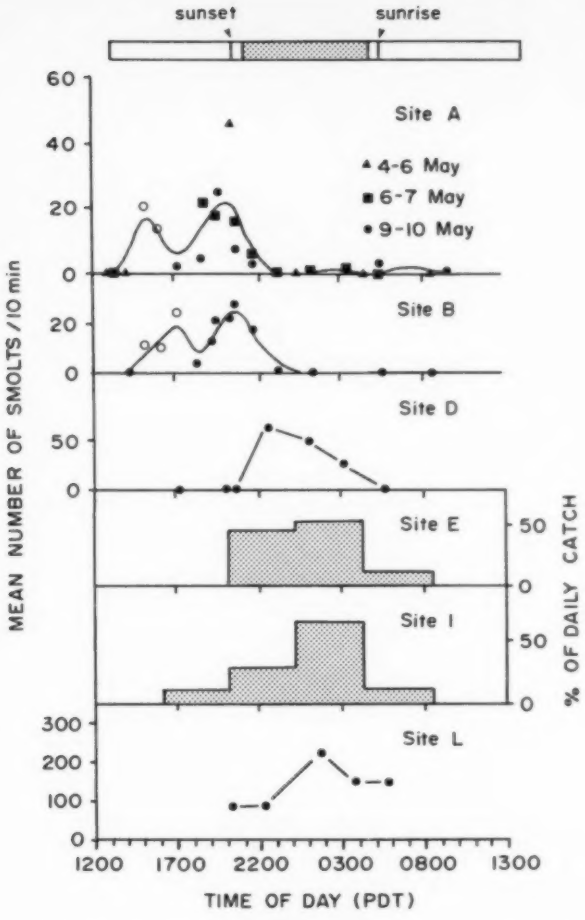

FIG. 4. Relative abundance of sockeye smolts at various sites in the Stamp and Somass rivers by time of day. Abundance at sites A and B was estimated using photographic techniques (4–18 May); open circles denote presence of sockeye smolts in schools that held position at the outlet without moving downstream. Abundance at sites D and L reflect underwater counts by divers on 25–27 April and 9–10 May, respectively. Abundance indices for sites E and I indicate the average percentage of total daily trap catches taken within 4-h sampling periods from 26 April to 12 May.

sockeye migration resembles a pulse that leaves Great Central Lake at dusk (20:00 PDT), reaching Stamp Lagoon (site D, 2.2 km downstream) by 01:00 PDT and the end of the Stamp River (site I, 17.7 km downstream) before 04:00 PDT. This implies that most smolts required less than 8 h to migrate 20 km from Great Central Lake to the upper Somass River, an average ground speed of 2.5 km/h.

Measurements of maximum water velocity at 0.5-km intervals between sites E and L on 16 June ranged from 1.4 to 8.7 km/h with a mean of 3.4 km/h. In other words, smolts drifting passively but maintaining position in the maximum current would have required about 6 h to travel the 20.6 km between sites E and L. Note, however, that the river level was about 0.4 m lower on 16 June than during late April and early May (Fig. 2). Water discharge in the Stamp River averaged 58.6 m^3/s (range 27.9–101.0) over the period in which 85% of smolts migrated (23 April – 25 May) compared with 41.8 m^3/s on 16 June (Water Survey of Canada 1990). Thus, maximum water velocity probably exceeded 3.4 km/h for most of the downstream migration.

Cross-correlations between daily catches at upstream and downstream sites (see Methods) also indicated that most smolts leaving Great Central Lake migrated past sites E and I in a single night. Daily variations in catches were significantly correlated between traps 2 and 3 only at a lag of 0 d (i.e., within the same day) (Table 1). Moreover, cross-correlations within the same day were positive and significant, or almost significant, for both the increasing phase ($r = 0.79$, $p = 0.06$) and the decreasing phase ($r = 0.57$, $p = 0.01$) as well as for the two phases combined ($r = 0.47$, $p = 0.02$).

Marine Migration

Corresponding patterns in the relative abundance and average size of smolts in purse-seine catches gave a generally consistent picture of sockeye migratory behaviour in Alberni Inlet and Barkley Sound. The first peak in abundance in Alberni Inlet was observed during the last week of April and first week of May which corresponded well to the timing of peak abundance during downstream migration (Fig. 5). A second peak in abundance was observed at several locations in Alberni Inlet about 1 mo later, in early June, which corresponded well to the second small peak in abundance during downstream migration. This peak was especially evident at trap 3 during the first week of June (Fig. 2), although we suspect that the size of the second peak was less than catches at trap 3 would suggest because of probable changes in the efficiency of trap 3 as the river level

TABLE 1. Cross-correlation coefficients (r) between detrended catches in traps 2 and 3 for time lags of 0–5 d. The number of data points (n) varies at each time lag because traps were not operated every day.

	Period of smolt migration								
Time lag (d)	Increasing phase			Decreasing phase			Phases combined		
	n	r	prob.	n	r	prob.	n	r	prob.
0	6	0.79	0.06*	18	0.57	0.01**	24	0.47	0.02*
1	3	—	—	12	0.14	0.67 NS	15	0.30	0.27 NS
2	2	—	—	8	0.20	0.64 NS	11	−0.03	0.94 NS
3	2	—	—	5	0.48	0.41 NS	7	0.35	0.44 NS
4	2	—	—	5	−0.69	0.20 NS	7	−0.48	0.28 NS
5	2			8	0.31	0.46 NS	10	0.18	0.63 NS

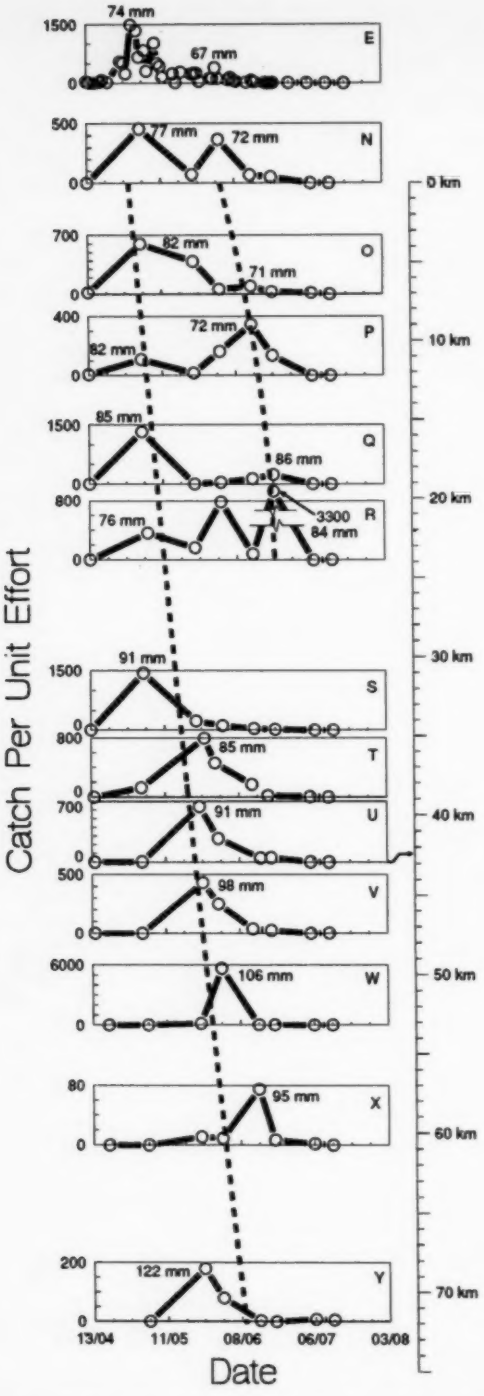

FIG. 5. Trends in juvenile sockeye abundance at trap 2 in the Stamp River (upper panel) and in purse-seine sets at 12 of the 24 sites in Alberni Inlet and Barkley Sound sampled between 13 April and 15 July 1989. Sites are denoted by letters in the upper right corner of each plot (see Fig. 1 for locations); distance from the first marine sampling location (Polly Point, site N) is indicated by the bar scale. The broken curves indicate a least-squares polynomial fit to the dates (in weeks from 13 April) of peak sockeye abundance with respect to distance (x, in km) from site N: date of first pulse = $2.25 + 0.070x + 0.000175x^2$; date of second pulse = $7.22 + 0.214x - 0.004039x^2$.

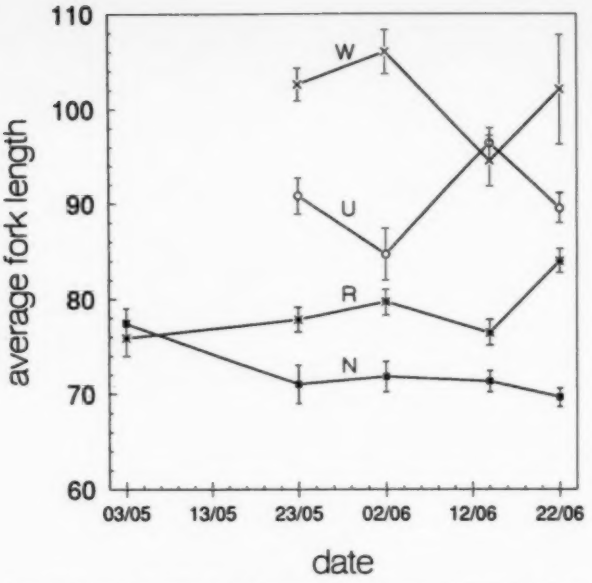

FIG. 6. Trends in average fork length (mm) of sockeye captured at two representative locations in Alberni Inlet (sites N and R) and in Barkley Sound (sites U and W) during April–June 1989. Error bars denote 95% CI for means.

declined. Peak abundance occurred several weeks earlier in Alberni Inlet than in Barkley Sound and in general occurred earlier at locations closest to Alberni Inlet (e.g., sites T, U, and V) and later at more distant locations (e.g., sites W and X).

The average size of sockeye smolts caught by purse seine at the marine sampling location closest to the mouth of the Somass River (site N) decreased steadily from 77.5 mm in early April to 69.5 mm in late July (Fig. 6). This decrease in size corresponded closely to that observed during downstream migration (Fig. 3) and reflects the fact that early migrants were typically larger than late migrants. However, the average size of smolts at the time of peak abundance at each site generally increased with distance from the Somass River (Fig. 5), as expected because of rapid growth during early sea life.

Speed of Marine Migration

We assumed that the date of the first peak in abundance at each seining location indicated the date of passage of the main pulse of sockeye smolts, corresponding to that observed on 2 May in the Stamp River. The second-degree polynomial plotted as a broken curve in Fig. 5 was the simplest model with the highest regression coefficient ($r^2 = 0.67$) of those examined, relating date of passage to distance travelled. Thus, an estimate of the "true" date of passage for the main pulse of migration can be read from the point where the fitted curve intersects the x-axis of each plot in Fig. 5. Based on this analysis, we estimated that juvenile sockeye maintained ground speeds of 1.9 km/d in Alberni Inlet during April–May and 1.6 km/d in Barkley Sound during the late May – July period. Only a few sockeye were captured in Barkley Sound in April and early

May. A similar analysis for the second pulse of migration (observed in the river around 2 June) indicated a slower speed of migration, about 1.3 km/d, for sockeye migrating through Alberni Inlet during June–July. The timing of the second pulse could be resolved more precisely than that of the first pulse at most locations owing to the increased frequency of purse-seine sampling in June–July.

Trends in average size of juvenile sockeye at seining locations varied with distance from the Somass River. The trend of decreasing size observed in the river (Fig. 3) and at site N in tidal waters was less obvious at sampling locations farther from the river mouth. In fact, it was reversed at some more distant sampling locations (Fig. 6).

Discussion

Sockeye migratory behaviour at the outlet of Great Central Lake was consistent with that reported by Groot (1965), Hartman et al. (1967), and Burgner (1991). Most smolts emigrated during May as expected, based on previously established relationships between the timing of smolt migration and latitude. Large schools gathered at the lake outlet just before sunset and began migrating downstream at dusk such that most (95%) had left Great Central Lake by 22:00 PDT. During darkness, the large schools fragmented into many small schools or solitary fish which either swam actively or drifted passively downstream depending on current velocity. Because these observations corroborate previous studies, it seems reasonable to assume that the migratory behaviour of Great Central Lake sockeye is fairly typical of other sockeye populations on Vancouver Island.

We found no evidence that sockeye smolts from Great Central Lake migrated downstream in daylight. Sockeye were observed in the river during the day only at sites G, K, and L, holding their position in slack water or in deep pools with back eddies. These smolts tended to remain in tight schools within several metres of the surface. The only other site in the Stamp River offering substantial slackwater habitat was site F which was not surveyed because access was difficult.

Several lines of evidence indicated that most smolts reached tidal waters within one night of leaving Great Central Lake. First, the average water velocity in the Stamp and Somass rivers was sufficient to carry smolts 20.6 km from the outlet of Stamp Lagoon to tidal waters in ≤6 h, provided they maintained position in the swiftest current (ground speed ≥3.4 km/h). Second, variations in daily catches were significantly correlated between upstream and downstream sites, but only within the same night. Third, peak abundance at observation sites progressively farther downstream resembled a pulse travelling at an average ground speed of 2.5 km/h (Fig. 4). At this speed, smolts from Great Central Lake would be expected to arrive at site L in the lower Somass River before 06:00 PDT. Similarly, smolts emigrating from Sproat Lake at 20:00 PDT and travelling downstream at the same speed would have arrived at site L, less than 8 km downstream, by midnight. This difference in predicted times of arrival at site L for Sproat and Great Central smolts is consistent with the broad peak in relative abundance observed at site L; smolt abundance rose sharply between 22:00 and 01:30 PDT but remained relatively high even as late as 05:00 PDT (Fig. 4). Note that because the migration timing and abundance of smolts from Sproat and Great Central lakes tend to be similar (Hyatt et al. 1990), it is probable that about half the smolts observed at site L had emigrated from Sproat Lake.

The fact that proportionately fewer large smolts (≥80 mm) were captured in trap 2 than in trap 3 suggests that smolt size was not sampled representatively at trap 2. Given that these large smolts migrated past trap 3 before midnight, they probably migrated past trap 2 before total darkness, and thus probably had greater opportunity to see and avoid the trap. Unfortunately, no smolts were collected at the outlet of Great Central Lake so that we cannot compare the size distributions of smolts emigrating from the lake with those from the traps farther downstream. However, no trends in smolt size or in the relative abundance of large smolts were evident in the photographs of smolts taken against flashboards at sites A and B at various times during the afternoon, evening, and night. Moreover, both large and small smolts were present in schools that held position at sites A and B during daylight (open circles in Fig. 4). If large smolts did not leave the lake earlier than small smolts, they must have travelled downstream more quickly than small smolts. This suggests that smolts swam actively for at least part of their downstream migration given that swimming performance increases with body length (Brett 1965). Even brief periods of active swimming would increase the speed of downstream migration by allowing smolts to escape back eddies and to maintain their position in the main current. Moreover, smolts must have swam across the still waters of Stamp Lagoon. We estimate that smolts required about 1 h to cross Stamp Lagoon on 27 April based on the relative timing of peak catches recorded at 2-h intervals for trap 1 (at the inlet) and trap 2 (at the outlet) and peak underwater counts in Stamp Lagoon itself. This implies a swimming velocity of roughly 1 km/h which is less than but approaching the physiological maximum sustained swimming speed of 5–7 body lengths/s or 1.3–2.0 km/h (Brett 1965).

In contrast with our observations in the coastal Stamp–Somass River system, other evidence indicates that sockeye smolts migrating downstream for more than 1 or 2 d in large rivers probably swim continuously once migration has been initiated. Anas and Gauley (1956) reported that marked sockeye smolts released from Winthrop and Leavenworth hatcheries on the upper Columbia River averaged 19–40 km/d downstream over distances of 560–670 km to the Bonneville Dam. In 1989, 32 "sockeye" smolts (probably outmigrant kokanee from Alturas Lake) were PIT-tagged and released from the Sawtooth Hatchery, Idaho, and later detected passing the Lower Granite Dam in the Snake River; they travelled 748 km downstream in 11.4–33.4 d (mean = 18.0 d), implying ground speeds of 22–66km/d (R. Kiefer, Idaho Department of Fish and Game, 1800 Trout Road, Eagle, ID 83616, personal communication). In 1990, 11 PIT-tagged sockeye travelled from Redfish Lake to the Lower Granite Dam (745 km) in 6.7–19.7 d (mean = 10.8 d), at ground speeds of 38–111 km/d, the faster passage presumably resulting from the higher water discharge in 1990 (R. Kiefer, personal communication). Maintained over 24 h, the rates of travel reported for Columbia River sockeye smolts imply typical ground speeds of 1.7–2.9 km/h (maximum = 4.6 km/h) which are very similar to those observed in the present study. Alternatively, if smolts held position during daylight in these rivers, they must have achieved typical ground speeds of 3.5–5.7 km/h (maximum 9.3 km/h), and it is necessary to postulate swimming speeds that seem implausible.

Times of passage of sockeye smolts past dams also suggest that sockeye smolts migrate continuously during day and night in the Columbia River. PIT-tagged sockeye smolts released from Cle Elum Hatchery in 1991 passed the McNary Dam

throughout the day with 59% passing in daylight (06:00–18:00 PDT) (data provided by the Pacific States Marine Fisheries Commission, Suite 200, 2501 SW 1st Ave., Portland, OR 97201). The mainstems of large rivers like the Columbia and Fraser are typically deep and turbid, and sockeye smolts are probably less vulnerable to predation during daylight in large rivers than they are in the Stamp–Somass system. Consequently, in large rivers, the advantages of decreasing transit time by migrating during daylight probably outweigh any increased risk of predation.

Sockeye smolts migrating downstream at night in the Stamp and Somass rivers were not especially vulnerable to predators. Most smolts were exposed to predators in the river for only one night, when their vulnerability to visual predators such as fish-eating birds (Vermeer 1989) and other salmonids (Wood et al., unpublished data) would have been redued. Sockeye smolts usually remained in midstream near the surface, thereby further reducing their vulnerability to large salmonids that were typically observed near the river bottom. Steelhead trout (*Oncorhynchus mykiss*) were the most abundant, large salmonids in the Stamp and Somass rivers, and these probably consumed <1% of the sockeye smolts migrating from that system in 1989 (Wood et al., unpublished data).

Purse-seine catches at most locations in Alberni Inlet and Barkley Sound gave a consistent indication of juvenile sockeye migratory behaviour. Evidently, most juvenile sockeye remained within the study area for about 6 wk (Fig. 5). Note that by swimming continuously at ground speeds of 1 km/h, juvenile sockeye could have travelled the entire length of Alberni Inlet (37 km) and across Barkley Sound (33 km) in <3 d. In general, the distribution, abundance, and migration timing observed in 1989 were similar to those reported in 1988 (Groot and Cooke 1988).

A few purse-seine catches did not fit the typical pattern. In Alberni Inlet, the major discrepancy was the unusually large number of sockeye (3300 fish) captured on 22 June at Bilton Point (Fig. 5, site R). This was the largest catch of juvenile sockeye at any location in Alberni Inlet from April to July, and it occurred many weeks after the main peak in downstream migration. Moreover, this large peak in abundance was not observed again at any more distant sampling locations. The timing of this large catch does correspond closely to the smaller (2 June) peak in abundance observed in the river and at several other locations in Alberni Inlet (Fig. 5). Thus, we speculate that this single large catch may have resulted from a temporary accumulation of juvenile sockeye owing to unusual physical or biological conditions (e.g., tides, wind, or food concentration).

The other major discrepancy in the purse-seine data is the apparent peak in abundance of juvenile sockeye on 25 May in Newcombe Channel in Barkley Sound (Fig. 5, site Y). This location, 76 km from the Somass River, was the most distant location sampled, and yet, inexplicably, catch rates there peaked at about the time of peak abundance at several other locations (e.g., sites W and X) that were much closer to the Somass River (approximately 30 km). However, catches at site Y were always small, and the average size of sockeye in the catch of 25 May was unusually large (122 mm), much larger than in any other catches from Alberni Inlet and Barkley Sound. Thus, these fish may have been very early migrants from the Somass River, or may have originated from Henderson Lake which is 35 km closer to Barkley Sound than either Great Cental Lake or Sproat Lake.

Although our marine sampling was too infrequent to determine peak dates of passage at each site with any precision, the overall pattern of abundance throughout the study area indicated that most sockeye migrated relatively quickly through Alberni Inlet then slowed down as they entered Barkley Sound (Fig. 5). The apparent dates of peak passage past all sampling sites indicated that the first pulse of smolts migrated through Alberni Inlet and Barkley Sound at speeds of 1.9 and 1.6 km/d, respectively. However, careful inspection of Fig. 5 suggests that the simple polynomial model probably underestimates the speed of migration through Alberni Inlet because sampling was infrequent during April–May. Indeed, with these data, we cannot rule out migration speeds through Alberni Inlet as high as 6–7 km/d, as reported for Fraser River sockeye moving through the Strait of Georgia (Groot and Cooke 1987) and for Bristol Bay sockeye (Straty 1974). Of course, this would imply a more dramatic reduction in speed as smolts entered Barkley Sound. Early (April–May) migrants also appeared to move through Alberni Inlet faster than late (June–July) migrants, requiring about 19 and 28 d, respectively, to reach Barkley Sound.

Trends in average size at seining locations presumably resulted from the combined effects of initial size (e.g., size at site N), the speed of migration, and growth during migration. Thus, if early migrants travelled faster through Alberni Inlet than late migrants, and if growth rate did not decrease over the migration period, then growth increments between sampling locations in Alberni Inlet would be larger for late migrants than for early migrants. This would tend to counteract and eventually reverse the initial decreasing trend in average size at progressively more distant sites. For example, the average size of sockeye at site R, near the outer end of Alberni Inlet, actually increased slightly from April to July (Fig. 6). Sockeye caught at locations in Barkley Sound still farther from the Somass River (e.g., sites U and W) showed no obvious trend in size between May and July. Trends in average size would have become obscured at the most distant locations in Barkley Sound (e.g., sites U and W) if early migrants tended to slow their migration once outside Alberni Inlet such that both early and late migrants tended to accumulate in Barkley Sound, perhaps because forage was more plentiful there (i.e., zooplankton biomass was higher; Forbes et al. 1989, 1990).

Sockeye smolts migrating through Alberni Inlet in 1989 suffered heavy mortality due to predation by Pacific hake (*Merluccius productus*), spiny dogfish (*Squalus acanthias*), and walleye pollock (*Theragra chalcogramma*) (Hargreaves et al. 1990). Most predation on sockeye occurred in Alberni Inlet during April–May, coincident with the period of peak abundance of juvenile sockeye. During the period of highest predation (1–12 May), 75% of hake caught in gill nets in Alberni Inlet had one or more sockeye in their stomachs (Hargreaves et al. 1990). Overall, these authors estimated that hake and other predators consumed up to 12 million juvenile sockeye, or more than 50% of the total production of sockeye from the Somass River in 1989. However, losses of juvenile sockeye to predators in Alberni Inlet declined dramatically during June–July.

Hake and other fish predators sampled in Barkley Sound in 1989 had not consumed any juvenile sockeye, apparently because they preferred to feed on alternate prey such as euphausiids and juvenile herring that were abundant in Barkley Sound, but rare or absent in Alberni Inlet (Hargreaves et al. 1990). Thus, the impact of these predators on sockeye in Barkley Sound was probably minimal, despite the slower migration rate. We speculate that the relatively rapid migration of juvenile

salmon through Alberni Inlet during April–May, and the subsequent slowing of the migration rate in Barkley Sound, and in Alberni Inlet during June–July, may have been a direct behavioural response to harassment by predators. In any case, we conclude that the relatively rapid migration observed during April–May compared with that during June–July would have reduced the overall mortality of juvenile sockeye caused by predation in the early sea life period.

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Factors Related to the Variation in Mixing Depth among Meromictic Lakes

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McGuire, S., and D. J. Currie. 1993. Factors related to the variation in mixing depth among meromictic lakes. Can. J. Fish. Aquat. Sci. 50: 1338–1342.

Although the factors that influence the mixing depth of holomictic lakes have been well investigated, meromictic lakes, which never mix completely, have received little attention in this regard. The purpose of the present study was to determine if the geographic variation in mixing depth of the mixolimnion is related to the same factors as is the epilimnion depth. Data on the lake morphometry, elevation, latitude, salinity in the mixo- and monimolimnia, and the depths of the epi- and mixolimnia were obtained for 56 meromictic lakes worldwide. The depth of the mixolimnion was most closely related to the maximum depth of the lake ($r^2 = 0.69$); no other variables were significant after accounting for depth. In the same data set, epilimnetic depth was also most closely related to maximum depth, rather than fetch, as earlier studies had found. Our results suggest that wind-induced turbulence has much less effect on the depth to which both the epilimnion and the mixolimnion circulate in meromictic lakes than does morphometry.

Si les facteurs qui influent sur la profondeur de mélange des lacs holomictiques ont été bien étudiés, les lacs méromictiques, qui ne se mélangent jamais complètement, n'ont reçu que peu d'attention à cet égard. L'objet de la présente étude était de déterminer si la variation géographique dans la profondeur de mélange du mixolimnion était reliée aux mêmes facteurs que la profondeur de l'épilimnion. Des données sur la morphométrie, l'altitude, la latitude, la salinité dans le mixolimnion et le monimolimnion et les profondeurs de l'épilimnion et du mixolimnion ont été rassemblées pour 56 lacs méromictiques situés dans le monde entier. La profondeur du mixolimnion était le plus étroitement corrélée à la profondeur maximum du lac ($r^2 = 0{,}69$); aucune autre variable n'était significative après la profondeur. Dans la même serie de données, la profondeur de l'épilimnion était aussi le plus étroitement corrélée à la profondeur maximale plutôt qu'au fetch, comme l'avaient montré des études antérieures. Nos résultats indiquent que la turbulence induite par le vent aurait beaucoup moins d'effets que la morphométrie sur la profondeur à laquelle l'épilimnion et le mixolimnion circulent dans les lacs méromictiques.

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Meromictic lakes are lakes that do not mix completely from top to bottom. This leads to a more or less permanent partitioning of the water column, where the upper layer, the mixolimnion, is usually aerated, while the water in the monimolimnion, the perpetually stagnant layer, is usually anoxic and more saline than the water above (Hutchinson 1957). A chemocline, which generally corresponds to a density gradient, separates the two strata. Worldwide, less than 200 of these lakes have been documented (Walker and Likens 1975; Croome 1986), but there are probably many more that remain to be described, especially among the saline lakes of Africa (McIntyre and Melack 1982) and the endorheic salt pans of central Asia. Meromictic lakes are found at all latitudes and on every continent (Hammer et al. 1978).

Mixing depth is an important feature of all lakes. Nutrient cycling and productivity, among other things, are strongly influenced by it (Straškraba 1980; Patalas 1984). In meromictic lakes, the mixing depth takes on particular importance due to its permanence. Because of the sharp delineation between aerobic and anaerobic environments, many aquatic organisms have vertical distributions that are determined by the chemocline. For instance, rotifers (Miracle and Vicente 1983) and bacteria (Croome 1986) have shown sharp stratification and extremely high density changes in relation to the chemocline.

In holomictic lakes, many studies have investigated the factors thought to influence the depth of the thermocline. Theoretical studies depict mixing depth as resulting principally from the counteracting influences of turbulence induced by wind and stability induced by thermal density gradients (Hutchinson 1957; Johnson et al. 1978). Relatively simple one-dimensional models can describe the average stratification conditions in individual lakes reasonably well (Imberger 1985). The three-dimensional fine structure of stratification within a lake can be very complex due to internal currents, variation in wind speed over the surface of the lake, basin morphology, atmospheric stability, etc. (Imberger and Hamblin 1982; Imberger 1985), and these factors may strongly affect the local distribution of materials within a lake.

For purposes such as modelling geographical variations in productivity (Straškraba 1980), factors affecting the internal structure of stratification in particular lakes are of less interest than the factors that influence the variation in average conditions of stratification among lakes. The bulk of the variation among lakes in mixing depth during the stratified period has been empirically related to morphometric factors that influence turbulence and heat transfer. Yoshimura (1936) showed that the

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TABLE 1. Means, variances, and ranges of the variables examined in this study. Z_{epi} is the thickness (m) of the epilimnion, Z_{mixo} is that of the mixolimnion, and Z_{max} is the maximum depth. S_{mixo} and S_{monimo} represent the salinities ($g \cdot L^{-1}$) of the mixo- and monimolimnia, respectively. Surface area was measured in km^2 and fetch in m. Mean annual air temperature (Temp.) is in °C. Common logs are shown because most of the variables are approximately log-normally distributed.

Variable	n	Mean	Variance	Minimum	Maximum
$\log(Z_{epi})$	41	0.779	0.084	−0.301	1.18
$\log(Z_{mixo})$	56	1.04	0.211	−0.046	2.12
$\log(Z_{max})$	56	1.42	0.227	0.415	2.55
log(surface area)	55	−0.352	1.72	−3.19	3.53
log(fetch)	52	0.154	0.632	−1.301	2.27
log(elevation)	55	2.09	0.893	−0.187	3.28
$\log(S_{mixo})$	34	0.535	0.887	−1.30	2.18
$\log(S_{monimo})$	33	1.37	0.542	−0.469	2.58
$\log(S_{mixo} - S_{monimo})$	33	1.10	0.832	−1.22	2.36
Temp.	56	7.2	89.7	−21.0	26.7

among-lake variation in epilimnetic depth is a power function of lake surface area. Patalas (1961, 1984) and Arai (1981) were able to predict thermocline depth in holomictic lakes as a power function of lake fetch.

In principle, similar considerations should determine the depth of the chemocline in meromictic lakes: stability is influenced mainly by gradients of heat and salinity, while turbulence is provided mainly by wind. As far as we are aware, no empirical studies have examined the variation in mixolimnetic depth in meromictic lakes. It is possible that different variables may be correlated with the depth of the chemocline in meromictic lakes, owing to the more complicated circumstances of stratification.

The primary purpose of the present study was to determine which environmental characteristics are most closely related to the depth of the chemocline in a worldwide sample of meromictic lakes and to compare these factors with the factors related to the depth of the thermocline. The secondary purpose was to develop models that could be used to predict mixing depth from data that are readily available from published sources. We considered both fetch and lake surface area, since they had previously been related to thermocline depth in holomictic lakes. Maximum lake depth was also considered, as morphometry may influence how effectively wind energy is transmitted to deeper waters. Climate seemed likely to be important, since it affects water temperature, and thereby water-column stability (Straškraba 1980). For example, warmer water temperatures have been associated with a shallower mixing depth in holomictic lakes (Patalas 1984). Mean annual air temperature was chosen for the present analysis, since it is closely related to water temperature (Straškraba 1985) and since it is easily determined from published maps. Latitude and elevation were used for the same reason. Salinity, by directly affecting the water density, greatly influences the stability of a column of water (Hutchinson 1957). Since a sharp density gradient is usually used as the indicator of the mixing depth of meromictic lakes, it was reasoned that the difference in salinity between the mixo- and the monimolimnia may also influence how deeply the water mixes.

Materials and Methods

Limnological and morphometric information on 56 meromictic lakes was obtained from the literature.[2] The lakes are distributed throughout the world, from 75°N to 77°S, and range in size from 0.0006 to 3420 km^2. The criterion for inclusion of a lake was that it had to have achieved total meromixis. Lakes exhibiting partial meromixis, where mixing occasionally extended to the bottom (e.g. for 1 or 2 d per year), were not included in the study.

In this study, the depth of the epilimnion was taken as the point of inflection of the temperature–depth profile. In principle, the depth of the mixolimnion could be analogously defined with respect to the salinity–depth profile. However, the salinity–depth profile was often irregular. We therefore took the bottom of the mixolimnion as the depth at which salinity (or associated chemical parameters) became detectably higher than in overlying waters. Mixolimnion depths were determined during the season of maximum mixing, usually autumn. The fetch was taken to be the sum of the longest distance between two points on the lakeshore, plus the widest perpendicular to that line.

Fetch, area, salinity, and the depths of the mixo- and epilimnia were determined from published figures when they were not otherwise given. Salinity in the mixolimnion was recorded as the average value (grams per litre) in that layer, and salinity in the monimolimnion was taken at the deepest recorded point. Elevation, when not given in the original sources, was estimated from topographical maps (Rand McNally 1988). The mean annual air temperature was determined using climatic atlases (Walter et al. 1975; Steinhauser 1979a, 1979b).

Certain data were missing from the data set. Some studies of meromictic lakes did not present summer thermal stratification data, so that it was not possible to estimate epilimnetic depth. Salinity data were also missing in several cases.

One lake in South Africa was excluded from the analysis (Ashton and Shoeman 1983). Its thermocline was reported as 4 cm deep, and it was described as a salt pan with a freshwater inflow. In a few cases, the depth of the epilimnion was apparently greater than that of the mixolimnion. In each of these cases, the chemocline was very sharp, while the thermocline was more gradual, such that the inflection point of the thermocline was located deeper than that of the chemocline. These data were retained in the analysis.

Results

The ranges of the variables studied are shown in Table 1. The depth of the mixolimnion was most strongly correlated with the maximum depth of the lake, while it was also significantly,

[2]The entire data set may be obtained, at a nominal fee, from the Depository of Unpublished Data, Document Delivery, CISTI, Building M-55, Montreal Rd., Ottawa, ON K1A 0R6, Canada.

TABLE 2. Simple linear Pearson correlations among the variables considered in this study. Variables include the depths of the epilimnion (Z_{epi}) and the mixolimnion (Z_{mixo}) and the maximum depth (Z_{max}), surface area, fetch, the salinities of the mixo- and monimolimnia (S_{mixo} and S_{monimo}) and their difference, latitude (Lat.), elevation (Elev.), and mean annual air temperature (Temp.). All variables except latitude were log-transformed prior to analysis. Correlations whose absolute values are >0.38 (shown in bold) are significant at $p < 0.05$. Others are not significant ($p > 0.05$).

	Z_{epi}	Z_{mixo}	Z_{max}	Area	Fetch	S_{mixo}	S_{monimo}	$S_{mixo} - S_{monimo}$	Lat.	Elev.
Z_{mixo}	**0.701**									
Z_{max}	**0.582**	**0.811**								
Area	**0.402**	**0.412**	**0.538**							
Fetch	**0.392**	**0.455**	**0.561**	**0.954**						
S_{mixo}	**0.389**	−0.146	−0.165	0.199	0.090					
S_{monimo}	**0.426**	0.090	0.004	0.283	0.170	0.577				
$S_{mixo} - S_{monimo}$	0.281	0.084	−0.026	0.236	0.162	0.366	**0.952**			
Lat.	0.029	0.250	0.283	0.136	0.178	−0.169	0.070	0.164		
Elev.	−0.209	−0.061	−0.221	−0.118	−0.071	0.077	−0.044	−0.030	−0.182	
Temp.	−0.089	−0.117	−0.135	−0.117	−0.158	0.140	−0.180	−0.156	**−0.869**	0.036

albeit less strongly, correlated with fetch and area (Table 2). The best model relating mixolimnetic depth and maximum depth is the following:

$$\log(Z_{mixo}) = -0.48\ (\pm 0.50) + 1.42\ (\pm 0.69)\log(Z_{max}) - 0.22\ (\pm 0.23)\log(Z_{max})^2$$

where Z_{mixo} is the depth of the mixolimnion (all depths in metres) and Z_{max} is the maximum depth of the lake ($n = 56$, $F = 56.3$, $p < 0.0005$, residual mean square = 0.070, adjusted $R^2 = 0.67$; 95% confidence intervals are given in parentheses). The quadratic term was retained in the model even though it was not quite significant ($p = 0.061$) because it reduced pattern in the residuals. After controlling for maximum depth, neither fetch, the difference in salinity between the mixo- and monimolimnia, nor any other independent variable accounted for additional variance when added to this regression.

The correlation between maximum depth and mixolimnion depth does not result simply from the fact that the mixolimnion is a nontrivial fraction of the total depth of the lake. The thickness of the mixolimnion is also strongly related to the thickness of the monimolimnion (Pearson $r^2 = 0.560$, $n = 56$, $p < 0.0005$), more strongly so than with fetch (cf. Table 2). The thicknesses of the mixolimnion and the monimolimnion have no logically necessary relationship with one another; their correlation cannot be a statistical artifact. This indicates that the mixolimnion is deepest in lakes where the lake as a whole is deepest, including the monimolimnion.

The variable most strongly correlated with epilimnetic depth in this set of meromictic lakes was also maximum depth (Table 2; Fig. 1). Once again, a quadratic function of log(maximum depth) was the best model describing epilimnetic depth:

$$\log(Z_{epi}) = -0.62\ (\pm 0.6) + 1.7\ (\pm 0.6)\log(Z_{max}) - 0.45\ (\pm 0.22)\log(Z_{max})^2$$

where Z_{epi} represents the depth of the epilimnion ($n = 41$, $F = 23.0$, $p < 0.0005$, residual mean square = 0.041, adjusted $R^2 = 0.52$). Although mixolimnetic depth was not related to any other variables after controlling for maximum depth, epilimnetic depth showed significant partial correlations with the logarithms of lake surface area ($r = 0.321$, $n = 40$, $p = 0.043$), elevation ($r = -0.325$, $n = 41$, $p = 0.038$), and the salinity of the monimolimnion ($r = 0.402$, $n = 28$, $p = 0.034$).

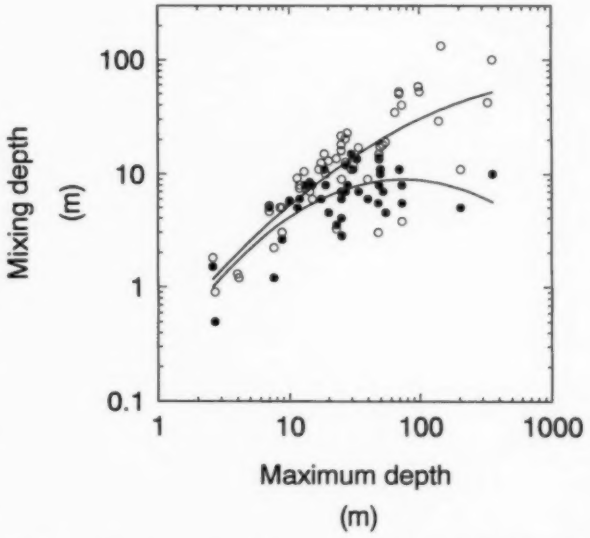

FIG. 1. Relationship between the maximum lake depth and the mixing depths of the epilimnion (solid circles) and the mixolimnion (open circles) in a worldwide set of 56 lakes. The lower line (for the epilimnion) and the upper line (for the mixolimnion) represent second-order polynomial regressions fitted to the data. The two relationships are highly significant and are statistically distinguishable.

The depths of the epi- and mixolimnia vary differently with maximum depth. In shallow lakes, the covariation is very similar (Fig. 1). However, as the lakes become progressively deeper, the depth of the epilimnion reaches a plateau whereas the mixolimnion becomes progressively deeper. A combined regression model, constructed to test for differences in the coefficients of the two polynomial models given above, shows that the intercepts and initial slopes of the two models do not differ, but the second-order term is significantly more negative for the epilimnetic depth than for the mixolimnetic depth (Table 3).

TABLE 3. Regression model relating epilimnion and mixolimnion depth, pooled together (Z_{layer}), to the maximum depth (Z_{max}) of the lake. A dummy variable (Layer) distinguishing between epilimnion depth (Layer = 0) and mixolimnion depth (Layer = 1) tests for differences in the regression coefficients associated with the two types of depth. All possible subsets of the following model were investigated: $\log(Z_{layer}) = c_0 + c_1 \cdot \log(Z_{max}) + c_2 \cdot [\log(Z_{max})]^2 + c_3 \cdot \text{Layer} + c_4 \cdot \text{Layer} \cdot \log(Z_{max}) + c_5 \cdot \text{Layer} \cdot [\log(Z_{max})]^2$. The model presented below had the smallest residual mean squared error (adjusted $R^2 = 0.673$, $n = 97$, $F = 66.9$, $p < 0.0001$, residual mean squared error = 0.057).

Predictor variable	Coefficient	SE	Student's t	p
Constant	−0.541	0.170	3.18	0.002
$\log(Z_{max})$	1.53	0.24	6.45	<0.001
$[1\log(Z_{max})]^2$	−0.260	0.080	3.27	<0.001
Layer·$[\log(Z_{max})]^2$	−0.129	0.019	6.61	<0.001

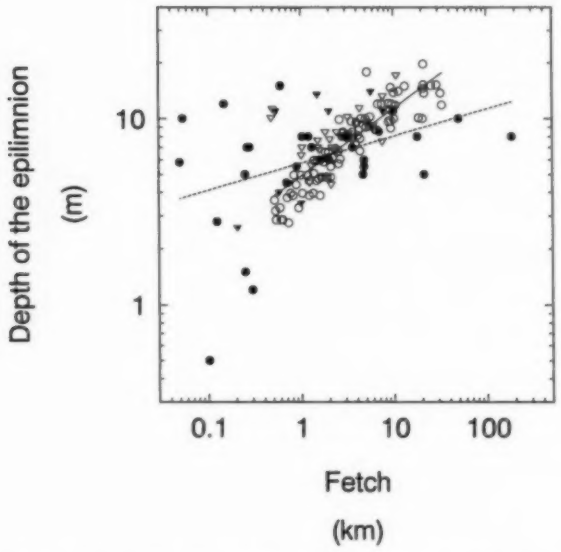

FIG. 2. Relationship between fetch and the depth of the epilimion. Open symbols represent a set of holomictic lakes in Canada and Poland (data redigitized from Fig. 2 and 3 of Patalas 1984). Solid symbols are from the present set of meromictic lakes. Circles represent lakes between latitudes 60°N and 60°S. Triangles represent lakes closer to the poles. The solid line and the broken line represent linear regressions to the holomictic and to the meromictic data, respectively.

Discussion

The maximum depth of lakes proved to be the best predictor of the depths of both the epilimnia and the mixolimnia of meromictic lakes. Patalas (1961, 1984) and Arai (1981) both used fetch to predict epilimnetic depth. In this study, although fetch was significantly related to both epilimnetic and mixolimnetic depth, the correlations with maximum depth were much stronger. The earlier studies did not investigate maximum depth, so it is impossible to say if maximum depth is a better predictor in those data sets as well.

These results lead first to the question of why we did not find fetch to be the best predictor of epilimnetic depth whereas earlier studies did. There are two possible explanations. Since neither the holomictic nor the meromictic data sets are random samples of lakes, peculiarities of the data sets may lead to correlations that are not general. Alternatively, fetch may indeed have less influence on mixing depth than does the maximum depth of the lake.

Our data set differs from those of Patalas (1961, 1984) and Arai (1981) in two respects: all of our lakes were meromictic whereas few (if any) meromictic lakes were included in earlier studies. Second, earlier studies were geographically quite limited (northern Poland, Canada, and Japan, respectively) whereas our set included lakes worldwide.

The relationship between fetch and epilimnetic depth in our study is statistically distinguishable from that observed in Patalas's (1984) data (Fig. 2). The effect of fetch on epilimnetic depth is much stronger in Patalas's set of Canadian and Polish holomictic lakes (slope = 0.375, SE = 0.062) than it is in the present worldwide set of meromictic lakes (slope = 0.187, SE = 0.036). This difference is significant even if one restricts our worldwide data set to the same range of fetch as that observed by Patalas. This suggests that there is indeed a systematic difference in the effect of fetch on meromictic versus holomictic lakes.

It is possible that the morphology of meromictic lakes reduces the importance of fetch, relative to holomictic lakes. Typical meromictic lakes in temperate zones are small and highly sheltered (Hutchinson 1957), and several of the African and Australian lakes in the present data set are maar (crater) lakes, which are also highly sheltered by the crater walls. This might reduce the importance of wind-induced mixing. However, our data set also includes lakes with greater fetch than did earlier studies of holomictic lakes. Further, some lakes, such as the Great Salt Lake, are very exposed. This suggests that reduced wind exposure is probably not the critical difference between our set of meromictic lakes and earlier sets of holomictic lakes.

It is also possible that depth assumes greater importance in meromictic lakes because maximum depth is more variable among the lakes in the meromictic data set than among lakes in the holomictic data set. Meromictic lakes are typically deep, although we have no data to test the hypothesis that they are deeper, on average, than Patalas's lakes.

The second obvious question posed by our results is why deep lakes stratify more deeply than do shallow lakes, even after controlling for fetch (the partial correlations between epi- and mixolimnetic depths with maximum depth after controlling for fetch are $r = 0.496$, $n = 40$, $p = 0.0011$ and $r = 0.728$, $n = 52$, $p < 0.0001$, respectively). An equivalent observation is that the proportions of the water column in the epilimnion or in the mixolimnion are much less variable than are the absolute depths of the epilimnion or mixolimnion. It is not clear why this should be the case. The depth of a lake, after controlling for fetch, is presumably unrelated to wind-induced tur-

bulence. Greater maximum depth could decrease stability by increasing the distance over which a stability gradient, be it generated by temperature or salinity, must be maintained. It is also possible that, in meromictic lakes, a given input of solute can maintain a permanent density gradient only over a particular volume of water, so that all else being equal, the mixing depth would be deeper in larger volume lakes. If this were true, one would expect mixolimnion depth to be even more strongly correlated with lake volume than with maximum depth. Our data do not permit a direct test of this hypothesis.

A third question arising from our results is why the thermocline is deeper in lakes with more saline monimolimnia (i.e., why there is a significant partial correlation between epilimnetic depth and monimolimnetic salinity, after controlling for maximum depth). When the monimolimnion is very saline, it is also often very warm; increased density due to the salinity more than compensates for decreased density due to higher temperature. Thermal stratification occurs at greater depths in saline systems, probably because heat transfer across the chemocline into the deep waters of the mixolimnion serves to decrease thermal gradients within the mixolimnion, thereby weakening thermal stratification and leading to greater mixing depth.

Patalas (1984) proposed that outliers from his model predicting epilimnetic depth as a function of fetch were due to climatic conditions. For example, he argued that a mild oceanic climate was the reason why one group of lakes mixed more deeply than his model predicted, although he did not test this hypothesis statistically. After digitizing the data from his study, we found that the depth of the epilimnion was indeed significantly related ($p < 0.0001$) to the two latitude classes that he established (>60°N and <60°N).

In contrast, in the present study, neither latitude, mean annual air temperature, nor elevation showed any significant relationship to the depth of either the epilimnion or the mixolimnion. The lakes used in this study comprised a much wider range of latitudes than in the studies for holomictic lakes, and all manner of climate types, from coastal arctic to alpine tropical, were represented. This suggests that factors that vary geographically on a worldwide scale influence mixing depth more strongly than the factors that vary across the range of latitudes in Canada studied by Patalas. For example, most of Patalas's lakes were on glaciated terrain, many of them on the Laurentian Shield (these lakes are typically shallow); in contrast, the global data set included lakes under a very wide range of depths and geological conditions.

Perhaps surprisingly, neither the salinity gradient across the chemocline nor the salinities of the mixo- or monimolimnia were significantly related to the depth of the mixolimnion in this study. It is not very likely that a larger sample of lakes would have revealed an effect of salinity on mixolimnion depth. The strongest correlation between the depth of the chemocline and any salinity variable is that with the mixolimnetic salinity: $r = -0.146$, $n = 34$, $p = 0.41$. Given these statistics, the probability that monimolimnetic salinity is in fact significantly correlated with mixolimnion depth, but that we have failed to detect the correlation, is $\beta = 0.246$.

Moreover, the absolute effect of salinity is very small: mixolimnetic salinity explains only 2% of the variability in mixolimnion depth, and the other salinity-related variables explain less. Salinity has been proposed as the main factor preserving the permanent stratification of meromictic lakes. (Hutchinson 1957; Wetzel 1975). A more explicit measure of stability of the chemocline might make better predictions of the interlake variation in mixing depth, but it is unlikely that the effect would be large. Density stability conferred by salinity gradients apparently influences mixing depth to a much lesser degree than do factors related to morphology. Similarly, temperature variables (mean annual air temperature, elevation, latitude) also did not explain significant amounts of variation in mixing depth.

In conclusion, it is clear that morphometry influences the variation in stratification depth among lakes to a much greater extent than do the factors thought to influence local and temporal variation in stratification depth (viz. exposure to wind and temperature- and salinity-induced density gradients). The mechanism by which morphometry influences mixing depth is not evident.

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LETTERS AND COMMENTS/LETTRES ET COMMENTAIRES

Potential Use and Abuse of General Empirical Models of Early Life History Processes in Fish

By necessity, the development of principles and theory in scientific research requires inferences based on previous observations. To summarize information from a variety of sources, some investigators advocate the development of general empirical relationships (e.g., Dillon and Rigler 1974; Banse and Mosher 1980; Peters 1983). General allometric models provide a series of qualitative and semiquantitative hypotheses about size-related features of organisms and in that sense hold promise for the advancement of knowledge and understanding (Peters 1992). However, many have questioned the potential value of empirical models in terms of their accuracy (Gulland 1987) and applicability to different levels of aggregation (e.g., McLaren and Corkett 1984; Lehman 1991) or on different time scales (e.g., McQueen et al. 1992). In this comment, we reiterate the cautionary suggestions of other investigators in the use of general empirical models, with particular reference to reviews of the early life history stages of fish (Miller et al. 1988; Pepin 1991). We both feel that there are potential gains and losses to our understanding that can result from using such empirical reviews. On the negative side, we find indications that such models can be used as predictive equations or as supporting evidence for underlying assumptions in specific studies. One possible implication is that estimates provided by general relationships could be substituted either into models or into field or laboratory studies as substitutes for the measurement of appropriate parameters. For this reason, we feel it important to stress the constraints of empirical reviews and warrant the need for verification at the appropriate level of observation or aggregation.

Because of the paucity of information on the ecological processes that influence the eggs and larvae of any one species of fish, several reviews have attempted to highlight general features of these stages of the life history (Dahlberg 1979; McGurk 1986; Anderson 1988; Miller et al. 1988; Bailey and Houde 1989; Houde 1989; MacKenzie et al. 1990; Pepin 1991). Empirical studies were surveyed with the aim of developing general conceptual frameworks that would unify observations on common variables (Miller et al. 1988; Houde 1989; MacKenzie et al. 1990; Pepin 1991). In most cases, the principal independent variable was the size of the organism because this feature is easily measured, available in most reports, and has been used extensively to generalize processes across taxa (e.g., Peters 1983). In studies of early life stages of fish, size is generally measured as length because of the difficulty in obtaining accurate measurements of weight, although some have found sufficient available information on weight (e.g., Houde 1989). In fact, weight may be the most appropriate descriptor of the size of organisms because of the significance of mass in determining metabolic requirements (e.g., Giguère et al. 1989). Despite the potential limitations of length as a measure of body size, the conclusions from general empirical reviews are consistent with previous expectations derived from simple physiological or ecological principles of allometry. For example, in comparisons between species, increases in size generally lead to increases in foraging efficiency (MacKenzie et al. 1990) and reductions in susceptibility to starvation (e.g., Miller et al. 1988) and vulnerability to predators (Folkvord and Hunter 1986; Pepin et al. 1987; Miller et al. 1988). These trends combine to increase the likelihood of surviving through a given stage of the life cycle of larger species (Pepin 1991). However, these conditions may not always be satisfied (see Pepin 1991). Given the consistency of these observations with basic expectations, one can easily be tempted to extend these interspecific relationships to intraspecific ones and conclude that increased size of any early life history stage leads to enhanced survival.

Variability in the characteristics of larval fish (e.g., growth or mortality rates) can be compared or estimated at organizational levels that range from species (e.g., Miller et al. 1988; Houde 1989; Pepin 1991) to populations (e.g., Hempel and Blaxter 1967) to cohorts (e.g., Oak and Houde 1987), to families e.g., Chambers et al. 1988), to individuals (e.g., Chambers and Leggett 1987; DeAngelis et al. 1991). The issues addressed by studies conducted on these different hierarchical levels of organization can be very different. In a comparison between species, one is typically addressing the question *"Do the features of different species represent differences in life histories?"* Deviations from the general pattern become important as potential examples of a response to evolutionary pressures. In contrast, a comparison of individuals generally asks *"Are survivors good or lucky?"* This question is increasingly common in fisheries research, as we attempt to understand whether recruitment is controlled by selective processes during the early life history (Fritz et al. 1990; Crowder et al. 1992). Data provided by such different perspectives represent measurements on very separate levels of aggregation. Failure to recognize differing levels of aggregation is equivalent to making the assumption that size dependencies are independent of the level at which they are estimated. This is often not the case. For example, size-dependent vulnerability to starvation varies as one changes the level of aggregation from species (Miller et al. 1988; Pepin 1991) to stocks of a species (Blaxter and Hempel 1963) to families within a stock (Chambers et al. 1989) and finally to individuals within a family (e.g., P. Pepin and J.T. Anderson, unpublished data). These contrasting patterns reflect differences in the balance of life history characteristics at each level of aggregation. A similar difference in size dependency can be found in the predicted likelihood that an individual larva would survive exposure to a predator. Pepin et al. (1992) found that within experimental enclosures, larger individual larvae suffered higher predation rates than smaller ones. However, when they compared experiments, Pepin et al. (1992) found that the cumulative predation rates increased as the mean size of a cohort of larvae decreased. In this instance, apparent differences in size dependency did not come from differences in life history features but rather from differences in the manner by which the effects of encounter, attack, and capture probabilities were

being integrated. Clearly, forecasting individual survival probabilities based on the concept that increasing the size of a larva reduces vulnerability to capture by a predator (Brownell 1985; Folkvord and Hunter 1986; Pepin et al. 1987; Miller et al. 1988; Fuiman 1989) and thus leads to a reduced likelihood of dying could have produced erroneous predictions (see Litvak and Leggett 1992; Pepin et al. 1992). Similar results can also be found in comparative studies of size-selective mortality of pelagic fish eggs either between (Pepin 1991) or within (Rijnsdorp and Jaworski 1990) species. Finally, a study of growth and mortality rates among species shows that these vital rates are positively correlated (Pepin 1991) but variations in cohort survival are likely to result from fluctuations in either growth or mortality about the general relationship (Ware 1975). Depending on the level at which information is averaged, the effects of size can appear different and in some instances opposite to those at a different level.

To date, reviews of early life history characteristics have generally used the coarsest level of aggregation — comparison among species (e.g., Miller et al. 1988; Pepin 1991). The intent of our respective approaches was not to provide definitive relationships describing the various processes that are elements of the survival equation but rather to explore potential generalities emerging from the suite of studies surveyed. The empirical functions produced from the various analyses are not predictive models per se that are applicable to all species and levels of aggregation. Instead, they represent advanced *null hypotheses* that require verification, support, or rejection in future studies and at different levels of organization (aggregation). We refer to general empirical models as advanced null hypotheses because they constitute a summary of our current knowledge rather than the basic null model of no significant relationship. These advanced null hypotheses provide a perspective that can be used to forecast an unknown species' characteristics. A comparison and test of observations from previously unstudied organisms can then be used to assess a species' departure from the expectation based upon the responses of many others. A proper analysis to assess the *practical* and *statistical* significance of the deviation(s) from the general empirical model can then lead to further hypotheses about differences in other life history features or variability in reproductive success (see Chambers and Leggett 1991 for further discussion). However, the patterns emerging from interspecies comparisons may not be robust when one changes the level of organization considered. Without further testing and verification, predictions assuming that bigger is necessarily better can lead to spurious or inaccurate conclusions.

Our proposals that size can be used to describe features of development, production, and survival (Miller et al. 1988; Pepin 1991) are only two in a suite of perspectives that use this easily measured feature of organisms to compare across levels of aggregation (e.g., Bluewiss et al. 1978; Banse and Mosher 1980). The approach has been shown to be useful in a comparison between studies of different processes, species, or organizational levels (e.g., Sheldon et al. 1977; Dickie et al. 1987). But the conclusions should not be applied as general principles regardless of experimental or survey design. Furthermore, despite the appealing usefulness of simple empirical relationships in modelling growth, mortality, or survival of fish eggs and larvae, it is imperative that we note the precision of such relationships. Data used to derive general empirical models often come from studies conducted at the population level for which measurements were taken at coarse space and time scales. The cumulative error associated with such measurements results in relationships that have broad confidence intervals (Miller et al. 1988, 1992; Pepin 1991). Consequently, the predictive power of models derived from these relationships is poor, and it is seldom possible to observe deviations either within or between species that are statistically discernable from the overall mean (Pepin 1993). General empirical models based on species comparisons provide first-order approximations to this process, but there is evidence that such coarse averages do not bear a strong association with adult abundance or future recruitment (e.g., Peterman et al. 1988).

In order to achieve an understanding of the mechanisms that determine overall survival, there is a need to develop a perspective that integrates information from several scales of observation (Crowder et al. 1992). By contrasting information on different scales of observations, we may be able to develop an understanding of how processes are being integrated relative to the population. General empirical relationships may provide the null models required to assess whether new information represents a departure from a summary of previous knowledge. However, when comparing observations from different sources, it is essential that we ask whether the accuracy and precision of the data are sufficient to detect deviations from a null model or an overall average. Although general models may serve as a tool to improve our understanding of early life history stages of fish, we feel they should not be used as substitutes for a lack of information pertaining to specific parameters — P. Pepin, *Department of Fisheries and Oceans, P.O. Box 5667 St. John's, NF A1C 5X1, Canada*, and T.J. Miller, *Department of Biology, McGill University, 1205 Avenue Dr. Penfield, Montreal, QC H3A 1B1, Canada.* (JB566)

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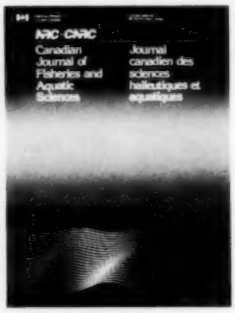
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